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*Acta Pædiat Scand 58* 1–9 1969

# INTRAVENOUS INFUSION OF GLUCOSE AND SODIUM BICARBONATE IN HYALINE MEMBRANE DISEASE

## *A Controlled Trial*

P O SAVIGNONI G BUCCI A CECCAMEA M MENDICINI
A SCALAMANDRÈ and M M ORZALESI

*From the Department of Pediatrics (Head A Colarizi) University of Rome Rome Italy*

The treatment of hyaline membrane disease (*HMD*) *of the newborn* by intravenous infusion of alkali and glucose proposed by Usher in 1959 (31) lies on sound theoretical grounds and is currently advocated (1 18 26) but there is no conclusive evidence of its efficacy In the only published study including a randomly selected control series diagnosis was established clinically (32) In other trials the treated and control series were obtained at different times or criteria for selection to experimental groups were not stated (5 9 14 15 17 32) in some studies there were no controls (16 30 33) and in several investigations chest X ray diagnosis was not included (see Table 6)

In the present controlled trial diagnosis was established by radiological criteria and there was random selection of treated and control patients The possible effects of treatment on neonatal mortality as well as on some clinical and biochemical parameters were evaluated In addition observations on the prognostic value of changes of systemic blood pressure in HMD are reported

## PLAN AND CONDUCT OF THE TRIAL SUBJECTS AND METHODS

All subjects who met the following requirements were regarded as eligible for the trial (1) Age less than [illegible] hours on admission (2) birthweight of 1 25–[illegible] 50 kg (3) Silverman score (25) higher than 2 (4) reticulo-granular pattern on chest film (5) no major congenital malformations or hemolytic disease All infants were born extramurally

Soon after admission to the Premature Unit clinical X ray and acid base studies were performed The chest film was immediately evaluated by two or more of the authors and if it was judged that granularity and/or diffuse hypotransparency were present the infant entered the trial The final interpretation of chest films was performed by a radiologist who had no information concerning the trial and subjects previously admitted were discarded if the typical reticulo-granular pattern was not recognized

The first 24 babies in the trial were assigned alternately to the treatment or control group Subsequently some changes in the experimental design were introduced In order to obtain final groups which could be compared with respect to arterial pH on admission babies were designated according to whether they had blood pH levels above or below 7 25 (approximately the median value in the 24 previously studied patients) and inclusion in either group was decided by means of sealed envelopes

It was decided to stop the study after 48 cases (24 treated and 24 controls) had been admitted to the trial

Treatment in all cases included incubator care at an ambient temperature of 32–34 C[1] administration of humidified oxygen at a concentration sufficient to produce as nearly as possible a normal arterial oxygen tension and one intramuscular injection of 3 mg of vitamin K (Konakion Roche)

All babies were given antibiotics until death or recovery from respiratory distress Control infants received intramuscular kanamycin (10 mg/kg/day)

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Patients in the treatment group were given intramuscular Kanamycin plus intravenous penicillin (100 000–300 000 U/day) in the first half of the study and intravenous methicillin (100 mg/kg/day) plus Colistin (8 mg/day) thereafter Kanamycin was never given for more than 10 days (in most cases for 5 days) and other antibiotics were substituted if treatment was still required Respiratory standstill was treated with artificial respiration by mask

Infants in the *control group* received no feeding on the first day of life 25 ml/kg/day of 10 glucose solution by mouth in the second day to which 25 50 75 and 100 ml/kg/day of pooled human milk were added over the next four days

Infants in the *treatment group* received into a peripheral vein a continuous infusion of 10 glucose solution (70 ml/kg/day) with sodium bicarbonate The initial dose of bicarbonate (mEq given = base deficit of blood mEq/l × body weight kg × 0 5) was given in 2–6 hours and a maintenance dose was administered thereafter with additional amounts if metabolic acidosis was still present or if metabolic alkalosis was required to compensate for respiratory acidosis Although the plan was to keep the arterial pH above 7 30 no attempts were made to correct completely for severe hypercarbia (i e Paco above 70 mm Hg) In two infants with extreme acidosis one third of the required amount of base was given as Tris buffer After the third or fourth day of life oral feeding with added potassium was administered

Clinical observations included repeated recording of a modified Silverman score (see under Results—clinical data on pulmonary function) respiratory rate and skin colour Gestational age was estimated from the first day of the last menstrual period and was rounded to the nearest week

The systolic blood pressure (SBP) was measured indirectly from the brachial artery by a modified xylol/pulse indicator instrument (4) The difference was also calculated between the observed values and normal average values predicted from body weight gestational age and post natal age (ΔSBP mm Hg = observed–predicted SBP) according to a multiple regression equation obtained in a previous study (8)

Antero posterior chest films were taken in duplicate by a portable X ray machine (focal distance 90 cm exposure time 0 06 sec intensity 50–55 kV) without removing the infant from the incubator

Acid base and oxygen determinations were performed on arterial or arterialized capillary blood by means of a micro Astrup apparatus and a Clark type micro electrode Carbon dioxide tension (Pco) oxygen tension (Po) and pH were corrected for the temperature of the baby Right to-left shunt was calculated from arterial Po when breathing oxygen at a concentration higher than 80 Additional details on methods of sampling measurements and calculations have been described elsewhere (6 21)

Statistical analysis of the results was performed by the following methods

Differences in average values were analyzed by the Student s *t* test (10)

Differences in mortality or in the prevalence of various situations were analyzed by the $\chi$ method (10)

Finally the survival curves of control and treated patients were analyzed by the Wilcoxon two sample test (or rank sum test) (10) assigning ranks to the survival time in dead infants and assuming a normal distribution for survivors of both groups

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Regression equation for SBP of healthy newborn premature infants (age 2–96 hours)

SBP mm Hg = 23 2 + 8 13 bw + 0 503 g a + 0 226 pn a − 0 0016 (pn a)

where bw = body weight (kg) g a = gestational age (weeks) and pn a = post natal age (hours) When the gestational age was not known we assumed the median value according to birth weight

## RESULTS

### *Population with RDS and pulmonary X-ray abnormalities observed during the trial period*

The trial started on March 1964 and ended on December 1966 During this period 95 newborn infants with birthweight of 1 25–2 50 kg RDS (as defined by a Silverman score higher than 2) and abnormal lung X ray findings were admitted within the first 24 hours of life (Table 1)

In 60 babies reticulo granularity was demonstrated on the chest film Forty-eight of these were included in the trial The remaining 12 were not included for various reasons (hemolytic disease acid base studies not available failure of laboratory equipment died immediately after X ray examination before acid base studies could be performed subgroups already completed)

Abnormal X ray findings (without granularity) were reported in 35 additional patients and included mild diffuse opacity of lung fields (91 ) patchy opacities (80 ) air bronchograms (37 )

From Table 1 it can be seen that mortality and frequency of pulmonary HMD on post mortem specimens were high in infants with a reticulo granular pattern and low in the remaining patients Ninety seven per cent of the 31 babies who died with HMD proven at autopsy had reticulo-granularity on chest films

Table 1 *Mortality and histological hyaline membrane disease (HMD) in newborns with RDS birth weight of 1 25–2 50 kg and abnormal lung X ray findings observed during the trial period*

| X ray findings | Controlled trial | Total no | Died no | Lung post mortem examinations No | | |
|---|---|---|---|---|---|---|
| | | | | No | No | c HMD |
| Reticulo-granularity | Yes | 48 | 33 (69 %) | 28 | 23 (82 %) | |
| Reticulo-granularity | No | 12 | 10 (83 ) | 8 | 7 (87 ) | |
| Total c reticulo-granularity | | 60 | 43 (72 %) | 36 | 30 (83 ) | |
| Abnormal no reticulo-granularity | No | 35 | 8 (23 ) | 5 | 1 (20 ) | |

*Comparability of experimental groups outcome and post mortem findings*

The treatment and control groups were reasonably similar with respect to characteristics and clinical and laboratory findings on admission (Tables 2 and 3) with the main exception of a higher percentage of males among controls

The age when infusion was started and the age of death in fatal cases is illustrated in Fig 1 Only two patients (cases nos 10 and 11) died within 12 hours of the beginning of treatment no trend of higher mortality in late treated infants was apparent

*In the* treatment group the mortality rate was lower than in controls (58 vs 79 ) but not significantly so ($p = 0.076$) As shown in Fig 2 early death was less frequent than in controls again an insignificant difference However th combined effect of lower mortality and longer survival of fatal cases was such that the survival curve of treated patients was significantly ($p < 0.02$) different from controls (Fig 2) No deaths occurred after the fifth day of life Among survivor marked chest retractions were observed beyond the fourth day of life in 50 of treated patients and never in controls In one treated survivors a right pneumothorax was successfully managed by continuous intrapleural suction at a pressure of $-11/-13$ cm H O

Post mortem findings have been summariz d in Table 4 Significant intracranial (subarachnoid and/or intraventricular) hemorrhage was found in 6 treated and 7 control patients

Table 2 *Characteristics of infants in the trial*

| | Treated | Controls |
|---|---|---|
| Total number | 24 | 24 |
| Birthweight kg | | |
| 1 25 1 50 | 12 | 8 |
| 1 51 2 00 | 10 | 11 |
| 2.01 50 | 2 | 5 |
| Gestational age weeks | | |
| 31 | 8 | 4 |
| 31 35 | 10 | 10 |
| > 35 | 2 | 2 |
| Unknown | 4 | 8 |
| Males | 12 | 19 |
| Cesarean section | 3 | 4 |

Table 3 *Clinical and laboratory findings (mean and range) on admission in infants in the trial*

| | Treated | Controls |
|---|---|---|
| Total number | 24 | 24 |
| Age on admission hrs | 5 8 | 4 2 |
| | (1 17) | (2 15) |
| Silverman score | 5 2 | 4 8 |
| | (3–8) | (3–9) |
| Respiratory rate min | 57 4 | 60 2 |
| | (34–95) | (44–79) |
| SBP mm Hg | 47 9 | 47 5[a] |
| | (33–75) | (34–65) |
| ΔSBP mm Hg | −6 1 | −7 2[b] |
| | (−16 0/+18 0) | (−18 3/+6 0) |
| Arterial acid base status | | |
| pH | 7 234 | 7 234 |
| | (7 00–7 34) | (7 03–7 40) |
| Base excess mEq/l | −J 0 | −9 0 |
| | (−22 1/0 0) | (−15 9/−1 8) |
| Pco mm Hg | 49 0 | 51 8 |
| | (25 3 83 6) | (28 5–103 2) |

[a] Twenty three observations
[b] Twenty two observations

Patients in the treatment group were given intra muscular Kanamycin plus intravenous penicillin (100 000–300 000 U/day) in the first half of the study and intravenous methicillin (100 mg/kg/day) plus Colistin (8 mg day) thereafter Kanamycin was never given for more than 10 days (in most cases for 5 days) and other antibiotics were substituted if treat ment was still required Respiratory standstill was treated with artificial respiration by mask

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where bw = body weight (kg) g a = gestational age (weeks) and pn a = post natal age (hours) When the gestational age was *not known* we assumed the median value according to birth weight

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could not be excluded Taking such limitations into account a beneficial effect of treatment appeared to be present in patients with very low pH on admission and in those without severe hypotension For example none of the seven controls with blood pH on admission below 7 20 survived as compared with two of the five treated babies All infants with severe arterial hypotension on admission (i e ASBP < −12 mm Hg) died irrespectively of treatment whereas in patients without severe hypotension the mortality rate was lower in treated babies when compared with controls (Table 5)

*Biochemical and clinical course in treated and control infants*

*Acid base balance* During the first 10 days after birth 183 determinations of acid base status were performed on "arterialized" capillary or arterial blood (4 umbilical 3 temporal 91 radial artery) Twenty seven additional measurements of base excess (BE) were obtained on capillary blood

The average course of pH BE and $P_{CO}$ in treated and control infants who died or recovered, is illustrated in Fig 3 When comparing survivors it can be seen that the average pH level of 7 38 was attained by treated patients on the second day and by controls on the third day of life Without infusion the pH was in the majority of cases below 7 25 on the first day of life and it increased to above 7 30 in the survivors only after the age of 48 hours In most treated infants the pH was kept above

Table 4 *Most relevant post mortem findings in infants in the trial*

| | Treated | Controls |
|---|---|---|
| No died | 14 | 19 |
| No of autopsies | 11 | 18 |
| No ( ) c intracranial hemorrhage | 6 (46 %) | 7 (39 ) |
| No of lung microscopic examinations | 11 | 17 |
| No ( ) c HMD | 9 (82 ) | 14 (82 ) |
| No c severe pneumonia | 0 | 1 |

One c pneumomediastinum

Table 5 *Mortality according to prognostic factors in infants in the trial*

| | Treated | | Controls | |
|---|---|---|---|---|
| | No | % died | No | % died |
| Sex | | | | |
| Males | 12 | 67 | 19 | 84 |
| Females | 12 | 50 | 5 | 60 |
| Birthweight kg | | | | |
| < 1 50 | 13 | 46 | 11 | 100 |
| > 1 50 | 11 | 73 | 13 | 62 |
| Respir rate on admission | | | | |
| < 60 min | 13 | 69 | 13 | 85 |
| > 60 min | 11 | 45 | 11 | 73 |
| ASBP (mm Hg) on admission | | | | |
| < −12 0 | 5 | 100 | 5 | 100 |
| −12 0/ −6 0 | 8 | 64 | 7 | 71 |
| > −6 0 | 10 | 30 | 10 | 70 |
| Blood pH on admission | | | | |
| < 7 20 | 5 | 60 | 7 | 100 |
| > 7 20 | 19 | 58 | 17 | 71 |

7 25 in the second 12 hours of life and above 7 30 after the first day In treated infants who died the average BE levels were only slightly lower than in treated survivors but on the second day of life the pH became definitely lower because of increasing respiratory acidosis In patients who died without infusion the acid base parameters did not change on the average after the early hours of life however in individual cases various patterns of change were observed notably a sudden drop in BE occurring terminally in several babies

Available data were not sufficient to ascertain if treatment could influence the course of right to-left shunt

*Clinical data on pulmonary function* The modified Silverman score was separated into a retraction score (xiphoid upper thoracic and intercostal retractions) and a dyspnoea score (nares dilated mouth opened and expiratory grunt) each of the two scores ranging from 0 to 6

In each group the retraction score increased or remained at high levels after the early hours of life and decreased after the

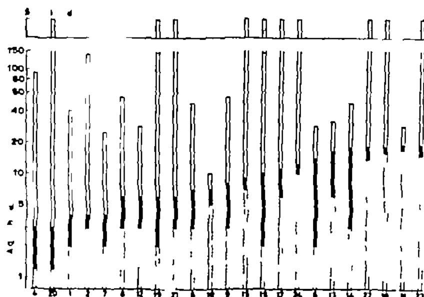


*Fig 1* Time from birth to admission (dotted column) from admission to start of treatment (black column) and survival time (white column) in 24 treated babies

Severe pneumonia was found microscopically only in one control and minimal lung infiltration in two The frequency of pulmonary HMD on post mortem examination was identical in the two experimental series

*The effect of treatment on mortality in various categories*

Several factors have been shown to be related to mortality in HMD (3 28) and it would be of interest to ascertain if treatment is likely to be more or less beneficial in particular categories of patients with different risks of death In an attempt to answer this question infants were divided in subgroups according to sex birthweight respiratory rate blood pressure and blood pH on admission For any given subgroup the number of cases and the mortality rate have been shown in Table 5 and the distribution of data on the remaining variants has been evaluated Gestational age and right to-left shunt were not considered because there were insufficient observations

In general mortality was lower in subgroups of treated patients than in corresponding control subgroups However the number of observations was small the observed differences were never statistically significant and in several comparisons interference due to unbalanced distribution of other factors with prognostic relevance

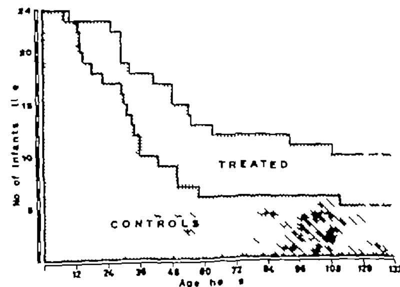



*Fig 2* Neonatal survival curves of treated and control infants The curves were compared by the Wilcoxon two sample test (10) and found significantly different ($p < 0.02$)

*Table 6 Published trials on alkali and glucose infusions in newborns with RDS (only studies including a control series have been considered)*

| References | Birthweight of subjects (kg) | Diagnosis | Experimental design | Total no of cases | Mortality % Treated | Mortality % Controls |
|---|---|---|---|---|---|---|
| Usher (32) | 0 9-2.5 | Clinical | Simult random | 70 | 17 | 37 |
| | 1 0-2 5 | Clinical | Not simult | 212 | 20 | 42 |
| Keuth & Adenauer (17) | 0 7-2 4 | Clinical | ? | 36 | 33 | 71 |
| Hutchison et al (14) | All weights | X ray | Consec | 232 | 46 | 64 |
| Iversen & Zachau Christiansen (15) | <2.5 | Clinical | Consec | 64 | 50 | 80 |
| Present study | 1 25-2 50 | X ray | Simult random | 48 | 58 | 79 |

Simult not simult consec = Time of treatment and control series (simultaneous not simultaneous consecutive) random = randomized assignments to experimental series

## DISCUSSION

Few would doubt that an attempt should be made to correct severe metabolic acidosis in newborn infants since it is well known that many functions of the body may be deranged by an abnormally high hydrogen ion concentration this is particularly true for babies with respiratory distress syndrome because it has been shown that a low pH causes profound pulmonary vasoconstriction (13 23) and may therefore aggravate the respiratory insufficiency

A review of previous studies (Table 6) shows a wide range of mortality rate both in treated and control patients Such discrepancies must at least in part be due to different criteria for diagnosis In the present investigation radiological criteria alone were highly successful in selecting among newborns with respiratory distress syndrome a population with a high risk of death and a high incidence of hyaline membrane disease at autopsy (Table 1) In our experience the accuracy of diagnosis was greatly enhanced by taking chest films in duplicate and by repeating the examination when questionable findings were obtained in the early hours of life

Other discrepancies in mortality rate may also arise from differences in perinatal care before admission to the Nursery in selection of patients referred for special treatment and in treatment itself Difficulties in comparing mortality figures from various Centers may be partly overcome by the evaluation of parameters with prognostic relevance (3 28) but again it should be considered that the weight of prognostic factors may not be the same in different populations[1]

In the present study a definitely beneficial effect of sodium bicarbonate was demonstrated since treated infants had a significantly improved survival curve However the difference in mortality between treated and untreated infants did not reach statistical significance showing that death is often caused by factors other than acidosis

Hypothermia may have contributed to increase the mortality rate in this series since for technical reasons it was not possible to raise the ambient temperature above 34 C and during the first day of life the rectal temperature remained below 36 C in 50 and below 35 C in 17 of the patients Infection did not appear a major cause of mortality since severe pneumonia was observed only in one of the 28 lungs examined microscopically indicating that differences in antibiotic treatment could not account for differences in mortality rate between experimental groups Intracranial he

[1] For instance by applying to our series the discriminant score for mortality published by Stahlman et al (28) we found that the mortality rate in subjects with a reticulo-granular pattern on the chest film was higher than predicted (actual 71% predicted 45%) while the reverse was true in babies with abnormal but not reticulo-granular chest X ray findings (actual 18% predicted 33%)

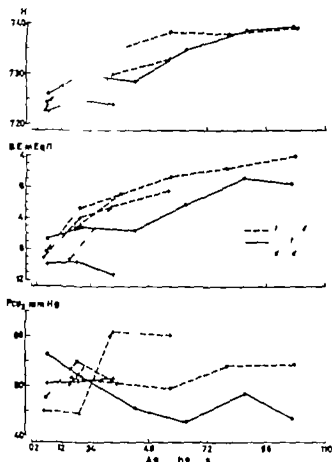


*Fig 3* Course of mean arterial pH BE and Pco in treated and control infants who died or recovered during the first five days after birth (treated patients at 2–12 hours only pre treatment values at 12–24 hours pre and post treatment determinations have been averaged separately after 24 hours only values after therapy)

second day The dyspnoea score decreased rapidly from the high values immediately after birth to practically nil after two days of age The respiratory rate attained maximum values between the 12th and the 36th hour of life with earlier and lower peaks in infants who died and with higher peaks reached at later ages in those who survived

When comparing survivors in the first 36 hours of life the retraction score was higher and the respiratory rate lower in treated babies The average retraction score remained higher in treated patients until the end of the fourth day of life although not significantly so Since in HMD mortality is inversely related to respiratory rate (28) and in view of the relationship between lung atelectasis and chest retractions these observations suggest that, in babies who survived after treatment, as a group pulmonary disease was more severe than in surviving controls

*Heart rate and systemic systolic blood pressure* No significant differences of average heart rate were observed between treated and control babies In one control patient, marked bradycardia due to 2 1 atrio-ventricular block associated with hyperkalemia and hypocalcemia (7) was observed in the second day of life which disappeared after Ca infusion but recurred shortly thereafter and persisted until death

The average course of systolic blood pressure (expressed as the differences from predicted normal values ΔSBP) in the various groups is illustrated in Fig 4 On average hypotension was present in each group at 2–12 hours of age in survivors both treated and controls, the SBP after a slight initial fall increased gradually to normal values by the fourth day of life when considering babies who died a transient increase of the average SBP was observed after the onset of treatment whereas in controls a further decrease occurred Death ensued in all 11 patients in whom a ΔSBP value below −15 mm Hg had been recorded some time during the disease and in 18 out of 22 babies with at least one ΔSBP value below −12 mm Hg

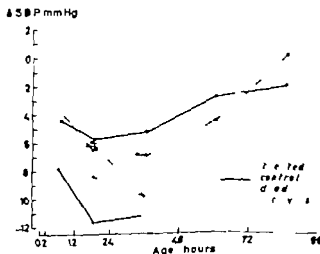



*Fig 4* Average course of ΔSBP in treated and control infants

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Other discrepancies in mortality rate may also arise from differences in perinatal care before admission to the Nursery in selection of patients referred for special treatment and in treatment itself Difficulties in comparing mortality figures from various Centers may be partly overcome by the evaluation of parameters with prognostic relevance (3 28) but again it should be considered that the weight of prognostic factors may not be the same in different populations[1]

In the present study a definitely beneficial effect of sodium bicarbonate was demonstrated, since treated infants had a significantly improved survival curve However the difference in mortality between treated and untreated infants did not reach statistical significance showing that death is often caused by factors other than acidosis

Hypothermia may have contributed to increase the mortality rate in this series since for technical reasons it was not possible to raise the ambient temperature above 34 C and during the first day of life the rectal temperature remained below 36 C in 50 and below 35 C in 17 of the patients Infection did not appear a major cause of mortality since severe pneumonia was observed only in one of the 28 lungs examined microscopically indicating that differences in antibiotic treatment could not account for differences in mortality rate between experimental groups Intracranial he

[1] For instance by applying to our series the discriminant score for mortality published by Stahlman et al (28) we found that the mortality rate in subjects with a reticulo granular pattern on the chest film was higher than predicted (actual 71% predicted 45%) while the reverse was true in babies with abnormal but not reticulo-granular chest X ray findings (actual 18% predicted 33%)

morrhage—still a poorly understood and possibly a major cause of death in RDS—was found in approximately 40% of patients and its occurrence was not dramatically modified by treatment

*The observation that all babies with markedly* decreased blood pressure on admission died *even when treated raises the question that* severe hypotension might produce death *per se* however this seems unlikely since many babies died after the blood pressure had returned towards normal following treatment Blood pressure has been shown to be low in infants with *RDS* by a number of authors (2 4 19, 20 24 27) but the reason for this finding is not clear although *there is some evidence that hypoxemia* and acidosis may produce hypotension (11 12 29) An interesting observation in this study was that, by using adequate reference standard for normal values early blood pressure changes were a factor of considerable prognostic importance in hyaline membrane disease


## SUMMARY

A controlled trial of the effect of intravenous infusion of glucose and sodium bicarbonate was performed in 48 low weight newborn infants with hyaline membrane disease

The experimental design included diagnosis by clinical and radiological criteria and random assignment to the treatment and control series

In treated infants the mortality rate was lower early death occurred less frequently and the neonatal survival curve was improved when compared with controls Only the difference in survival curves of treated and control patients was statistically significant

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We conclude that intravenous infusions of glucose and sodium bicarbonate should be part of the routine therapy of infants with hyaline membrane disease



## ACKNOWLEDGEMENT

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## ADDENDUM

*After this paper had been accepted* G Rowe & E K Cotton (*Pediatrics 41* 1063 1968) *found that* in infants with RDS the effective pulmonary blood flow rose and the right to-left *shunting diminished fol* lowing the rapid intravenous injection of sodium bicarbonate Similar observations had been *reported by* J M Gupta G W Dahlenburg and J A Davis (*Arch Dis Child 42* 416 1967) following administra *tion of amine buffer* THAM

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Table 1 *Idiopathic aplastic anaemia in children*

| Cas | Age at start of treatment (yrs) | Blood and marrow at start of therapy Hb (g/100 ml) | Ret ( ) | Granulocytes per mm | Platelets per mm³ | Bone marrow hypoplasia |
|---|---|---|---|---|---|---|
| R M | 2 5 | 4 9 | 0 4 | 200 | < 10 000 | Severe |
| A K P | 4 5 | 6 5 | 0 3 | 210 | < 10 000 | Severe |
| H H | 4 | 5 3 | 0 2 | 80 | < 10 000 | Severe |
| C D | 6 | 6 7 | 0 2 | 0 | < 10 000 | Moderate |
| A S | 7 5 | 4 4 | 0 2 | 140 | < 10 000 | Severe |
| H A Y[b] | 7 | 3 8 | 0 4 | 230 | < 10 000 | Severe |
| C R | 9 5 | 4 6 | 0 7 | 100 | < 10 000 | Severe |
| J M | 11 | 4 9 | 1 0 | 220 | < 10 000 | Severe |
| H K | 2 | 4 0 | 1 5 | 700 | < 10 000 | Moderate |
| M L | 11 | 7 5 | 1 6 | 700 | < 10 000 | Mod rate |
| E T | 3 | 3 4 | 1 4 | 1000 | < 10 000 | Moderate |
| A L | 3 5 | 9 5 | 5 2 | 1800 | 85 000 | Mod rate |
| M K[b] | 4 | 9 6 | 1 | 1300 | 30 000 | Moderate |
| L I | 6 | 6 7 | 0 | 200 | < 10 000 | Severe |
| B A | 8 | 9 9 | 5 | 1500 | < 10 000 | Moderate |
| G M S | 9 5 | 10 0 | 0 9 | 700 | 80 000 | Moderate |
| G O | 10 | 7 3 | 5 4 | 1300 | 12 000 | Moderate |
| M S (girl) | 14 | 8 4 | 5 | 240 | 15 000 | Moderate |
| M S (boy) | 14 | 4 5 | 0 5 | 690 | 20 000 | Moderate |

[a] Chloramphenicol [b] Siblings

5–10 mg a day if the bleeding tendency was controlled The androgen hormone was ad ministered parent rally to one patient as testo- *sterone propionate (1 7 mg/kg/day)* as sup- positories of testosterone Rektandron® to six patients at a daily dose of about 3 mg/kg (2 2– 4 5 mg) and to the remaining twelve patients orally as methyltestosterone in daily doses of about 2 mg/kg (1–2 3 mg)

The patients in the first group received the combined hormone therapy until they died The remaining patients were as a rule switched over to the less virilizing *metandienon (Dianabol®)* instead of testosterone when a satisfactory level of haemoglobin (11 *g or more) had been* reached At about the same time the corti costeroids were withheld Eventually metandie non was discontinued in those cases who main tained normal or nearly normal values even of granulocytes and platelets

## RESULTS AND COMMENTS

The course and treatment for all patients are demonstrated in Fig 1 It is obvious that the interval between the estimated beginning of the disease and the correct diagnosis varied considerably In four patients idiopathic throm bocytop nic purpura was diagnos d initially and two of th m even und rwent splenectomy Acute leucemia was the presumed diagnosis in three patients In another three cases the rela tively increased erythropo sis with maturation *d sturbances led to the* assumption of a fairly good haematopoetic activity combined with an *increased haemolysis* However on reexamina tion of the initial bone marrow smears it was evident that the megakaryocytes were lacking in all samples and that the granulocytopoesis was scanty with a relative increase of immature forms

Eleven of our nineteen patients responded initially to the combined treatment but the interval betwe n the start of therapy and the achievement of a satisfactory blood picture was very different The extremes were represented by *A L and G M S* who had an almost complete remission after two months and L I who re quired almost two years of continuous com bined treatment until a satisfactory remission

*Acta Pædiat Scand 58* 10–14 1969

# IDIOPATHIC APLASTIC ANAEMIA IN CHILDREN

## *Results of Androgen Treatment*

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The prognosis of untreated aplastic anaemia is poor at all ages In children the outlook has *improved considerably following* the introduction of the combined corticosteroid testosterone therapy by Shahidi & Diamond (12) Since then several authors have reported total or partial remission in about half of the children suffering from idiopathic aplastic anaemia (2 5 9 10) The rarity of the disease reflected by the small numbers described means that most paediatricians do not have the opportunity to diagnose and to treat more than a few cases Therefore we feel it is worth while to report our experience of 19 patients with idiopathic aplastic anaemia collected between 1959 and 1967 A preliminary report on 13 of these patients has been published earlier (6) Special attention has been paid to diagnostic difficulties and to factors of prognostic significance Treatment with anabolic steroids will also be discussed as well as the risk of relapse after cessation of hormone therapy

## MATERIAL

The series consisted of nineteen patients seven boys and twelve girls two to fourteen years of age Most of the patients were referred to us from other hospitals in Sweden Two children (the girl H H and the boy M S) were treated at their home hospitals according to the principles adopted for the rest of the series Patients with congenital malformations or retarded growth (4) were not included Since these patients do not show any definite improvement of the anaemia without therapy it is generally agreed that they shall receive continuous treatment

The diagnosis was based on the findings of pancytopenia in the peripheral blood and a moderately to severely hypoplastic or aplastic bone marrow in the absence of significant lymphadenopathy or enlargement of the liver and spleen The bone marrows were classified as severely hypoplastic when only lymphocytes reticulum cells plasma cells some mast cells and solitary erythroblasts were seen The most characteristic feature of moderate hypoplasia was the virtual absence of megakaryocytes Only a few white cell precursors were seen but relatively many erythroblasts Maturation disturbances were common In all patients representative bone marrow smears were investigated and also in most cases sections of formaline fixed bone marrow particles

Relevant findings at the start of the androgen therapy are presented in Table 1 The patients were divided into three groups according to the effect of therapy Eight patients who died without response constituted the first group The second was composed of two patients who died after an initial satisfactory response The remaining nine children showed considerable improvement after therapy and are alive after varying periods of time

The etiology was completely unknown in twelve patients Chloramphenicol was thought to be responsible for the aplasia in four instances

Two cases (H Å K and M K) were siblings without signs of malformations or retarded growth Since no environmental cause of the anaemia was found they possibly belonged to the group of constitutional aplastic anaemia first described by Estren & Dameshek (3)

## TREATMENT

All patients were treated with both testosterone and corticosteroids as suggested by Shahidi & Diamond (12) The corticosteroids were given as prednisone with initially high doses (40–80 mg per day corresponding to 2 mg/kg) this was gradually reduced during 3–6 weeks to

Table 2 *Initial blood values and the response to combined hormone therapy*

| | No response | Response |
|---|---|---|
| Reticulocytes > 1.0% | 0/8 | 8/11 |
| Granulocytes > 300/mm | 0/8 | 9/11 |
| Platelets > 10 000/mm | 0/8 | 6/11 |
| Moderate hypoplasia of the marrow | 1/8 | 10/11 |

tion of a second remission following a relapse of the disease as in the girl M S In view of the serious outlook for children with aplastic anaemia without androgen therapy (13 15) and the fact that more than half of the patients treated with testosterone may survive we feel that this substance should be used for the initial therapy together with corticosteroids

It is generally held that the rate of remission may be slow and as a rule several months will elapse until a normal haemoglobin concentration is reached However the absence of a response in the form of a decreasing transfusion demand a reticulocytosis and an increasing haemoglobin concentration during the first 2 to 4 months does not exclude a satisfactory result later on This is illustrated by the three year-old twin boy described by Best (1) who needed 18 months of combined therapy to achieve a normal haemoglobin level The girl L I of this series who required 23 months of testosterone and prednisone treatment is another example The practical consequences of these findings are that children with aplastic anaemia should be treated with prednisone testosterone until they die or recover The length of treatment and the side-effects are of secondary importance

The side-effects of the androgen treatment disappeared almost completely during the few months following the replacement of testosterone by metandienon The girl M S now 21 years of age had normal menstruations on 5 mg of metandienon per day When this treatment was discontinued she relapsed as shown Fig 1 During the treatment for remission ... found that 10 mg of metandienon produced amenorrhoea deeping of the voice and increasing hirsutism Following reduction of the daily dose to 5 mg the menstruations again became normal but the voice remained deeper than before

By dividing the series in those who responded and those who did not (Table 1) some information can be obtained regarding the prognosis In this series it was evident that severe pancytopenia combined with severe bone marrow hypoplasia were prognostically serious signs (Table 2) Thus all the non responding patients had severely hypoplastic marrows at the start of therapy except A S Unfortunately her marrow specimens were not examined histologically There were no signs of response in the marrow at autopsy in any of the patients of this group The majority of the patients in the response group had more than 1 reticulocytes and more than 300 granulocytes and 10 000 platelets per mm³ All cases but one also showed moderate hypoplasia of the bone marrow The girl L I with severe hypoplasia required androgen treatment for a longer period than the other patients in this group Thus it seems that the presence of some haematopoietic activity even if scanty is the most favourable prognostic sign which agrees with the findings of Shahidi & Diamond (13) and Lewis (8)

Two patients respond d initially but died later One case *H K* who was the first testosterone treated child in the series was sent home without maintenance treatment after the remission The subsequent relapse was recognized too late for institution of effective therapy The second patient M L while in good remission on prednisone and metandienon treatment suddenly developed a state of circulatory collapse after two days of a seemingly benign upper respiratory infection Treatment with antibiotics corticosteroids and transfusions was without effect and she died eight hours after admission to the hospital At autopsy a right lower lobe pneumonia was found as well as bilateral cortical atrophy of the adrenals The bone marrow of the vertebrae contained [illegible] cell rich blood forming tissue mixed

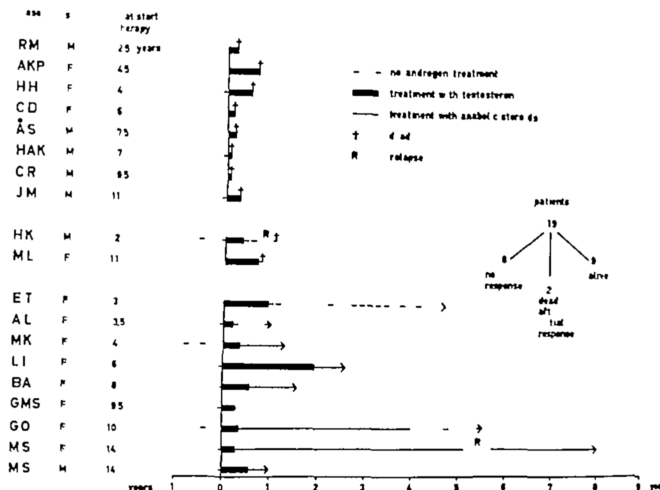



*Fig 1* Results of combined testosterone and prednisone treatment in 19 children with idiopathic aplastic anaemia

was produced In the first two cases the platelets had *started to rise immediately before the* androgen therapy Thus it is possible that a spontaneous remission coincided with the hormone therapy

In only three children (E T A L G O) was it possible to discontinue all therapy without signs of relapse during an observation period of ten to fifty-four months One patient the girl M S in whom treatment was stopped after five years gradually developed a relapse during the following six months However an almost complete remission was produced following a course of metandienon It is not clear whether this patient represented an atypical case of constitutional aplastic anaemia although no malformations were found on repeated investigations Growth retardation before or during therapy was not observed in any case of this series Shahidi & Grigler (14) did not find *any evidence of abnormally accelerated skeletal* maturation and no persisting endocrine abnormalities in three children on combined hormone therapy for aplastic anaemia This was thought to be due to the corticosteroids opposing the effect of testosterone

The successful use of some less virilizing anabolic steroids instead of testosterone at the start of therapy has been described by some authors (5 7 9 11) However no systematic comparison between the effects on aplastic anaemia of testosterone and anabolic steroids have been published Such a study would certainly be most difficult to perform in view of the rarity of the disease and the varying therapeutic response The present results indicate only that anabolic steroids may be used for the maintenance of and possibly also for the induc

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with atroph reas containing fat and a few reticulum c

It is ob us from the figure that some patients V I P C D A S and C R developed severe hypoplasia and pancytopenia in a very short time from the estimated beginning of the disease Probably these cases represented a more acute and serious form of aplastic anaemia which would not have responded even if treated at the start of the disease On the other hand in cases R M H H H A K and J M in whom the correct diagnosis was delayed three to seventeen months, the outcome might have been different if early androgen treatment had been instituted This supposition was supported by the fact that the bone marrows from the pre androgen period of these patients still contained haematopoietically active tissue Thus it may be concluded that every effort should be made to diagnose the disease before the bone marrow changes have progressed to severe hypoplasia

## SUMMARY

The results of treatment of 19 children with idiopathic aplastic anaemia is reported Initially the patients received testosterone-prednisone therapy When a satisfactory haemoglobin level had been reached metandienon was substituted for testosterone and the corticosteroids withheld 8 patients died without signs of response 2 patients responded initially but died later The remaining 9 patients are alive but in only 3 of them was it possible to discontinue all therapy without signs of relapse The rate of remission varied considerably One patient required 23 months of combined therapy to achieve a normal haemoglobin level A moderate hypoplasia of the bone-marrow at the start of therapy was the most favourable prognostic sign in this series Therefore it may be concluded that every effort should be made to diagnose the disease before the bone-marrow changes have progressed to severe hypoplasia

## ACKNOWLEDGEMENT

We wish to express our sincere thanks to many colleagues for their invaluable help in studying these patients

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# THE RELIABILITY OF THE DIAGNOSIS OF METACHROMATIC LEUCODYSTROPHY BY PERIPHERAL NERVE BIOPSY

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In recent years examination of a piece of peripheral nerve obtained by biopsy has become generally accepted and widely used for the diagnosis of metachromatic leucodystrophy (MLD sulphatidosis) (14 34) The various methods applied for histochemical demonstration of sulphatides include several metachromatic staining techniques (9 15) and fluorescence microscopy with acriflavine or trypaflavine as fluorochrome (16 17 18) With these methods sulphatide deposits can be demonstrated in peripheral nerves from cases of MLD not only in Schwann cells (7 35) but also in perivascular phagocytes (14 23 35) Staining with cresyl violet acetic acid (15) has been the recommended method and the one most frequently used

During the years since the method was first used in our laboratory it has been noticed that considerable amounts of brown metachromatic lipid may occur in peripheral nerves even in cases which autopsy does not prove to be MLD

Obviously more information is needed about the occurrence amount and distribution of histochemically demonstrable sulphatides in peripheral nerves in various conditions This information is necessary for making a differential diagnosis between MLD and other diseases affecting peripheral nerves

The present comparative study of histochemically demonstrable sulphatides in peripheral nerves has been made on cases belonging to three different categories i e cases of chemically and post mortem verified MLD cases with other diseases affecting the peripheral nerve and cases without clinical or morphological evidence of neurological disease

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## MATERIAL AND METHODS

### *Clinical material*

Peripheral nerves from 45 subjects were obtained through biopsy or autopsy (Table 1) The clinical material was divided into three groups

I The first group comprises seven clinically chemically and histopathologically typical cases of metachromatic leucodystrophy Clinical data concerning these cases have been presented previously (12) In addition a sural nerve biopsy has been included from one case (case no 8) with a typical history characterized by progressive motor and mental retardation, muscular hypotonia ataxia non functional gall bladder positive metachromatic urinary sediment and reduced conduction time in peripheral nerves The duration of the disease was 1 / years

II The second group consists of cases with clinical and morphological signs of peripheral nerve injury not due to metachromatic leucodystrophy The group includes the following diseases

Table 1 [illegible] ical composition of the material used for the histopathological investigation

| Group no | ( ) | Diagnosis | Age |
|---|---|---|---|
| I | 1 8 | Metachromatic leucodystrophy (1-7 verified post mortem) | 3-7½ years |
| II | 9 13 | Globoid cell leucodystrophy | ½-3 years |
| | 14-17 | Juvenile amaurotic idiocy | 16-21 years |
| | 18 | Infantile amaurotic idiocy | 4 years |
| | 19-29 | Diabetes mellitus | 31-62 years |
| | 30-31 | Uremia (chronic pyelonephritis) | 42-55 years |
| | 32 | Uremia (polyarteritis nodosa) | 34 years |
| | 33-35 | Carcinoma | 55-63 years |
| III | 36 | Abortion | 27 wks *in utero* |
| | 37 | Prolapse of umbilical cord | 0 |
| | 38-39 | Congenital heart disease | 3-4 days |
| | 40 | Intestinal atresia | 9 days |
| | 41 | Aspiration | 2 months |
| | 42 | Congenital heart disease | 4½ months |
| | 43 | Intestinal invagination | 8 months |
| | 44 | Aneurysm of coronary artery | 17 years |
| | 45 | Traumatic injury to ductus choledoccus with icterus | 30 years |

1 *Globoid cell leucodystrophy* (GLD Mb Krabbe) The histological and lipidhistochemical alterations in peripheral nerves in these cases have been previously reported (31 32) They were characterized by severe lesions of axons and myelin sheaths and accumulation of a substance which gave a positive staining reaction with Adams method for cerebrosides (1) No sudanophilic products were present

2 *Juvenile amaurotic idiocy* (Batten–Spielmeyer–Vogt disease BSVD) Also in these cases the peripheral nerves showed axonal and myelin changes with reduced stainability for the lipid components of the myelin sheaths and injuries to the axons There were no sudanophilic products (21)

3 *Infantile amaurotic idiocy* (Tay–Sachs disease TSD) In one case of TSD several spinal nerve roots were examined Detailed data on this case have been published by Eeg Olofsson *et al* (8) and Kristensson *et al* (21)

4 *Diabetes mellitus* All the cases examined revealed severe peripheral neuropathy which in some nerves had led to a complete destruction of the myelin sheaths and severe axonal damage (24) Prominent lipid histochemical features were reduced stainability for myelin lipids and lack of sudanophilic breakdown products

5 *Uremia and cancer cases with histological signs of a severe peripheral neuropathy* In contrast to the cases of diabetes the changes of myelin sheaths and axons in these cases indicated active decomposition in the form of drop shaped disintegration and accumulation of small amounts of sudanophilic breakdown products in two of the three cases examined

III This group comprises 14 control cases without clinical or morphological signs of peripheral nerve damage

## Histotechnical methods

The nerves were fixed in 10 per cent formalin Both frozen sections and paraffin sections were made The following methods were used

A *Frozen sections* The following procedures were applied on longitudinal sections 10–13 microns thick

1 0 1 cresyl violet dissolved in 1 acetic acid (pH about 2 7) (v Hirsch & Peiffer) to demonstrate brown metachromatic substance Sections which stained poorly at pH 2 7 were also treated at pH 4 This pH was obtained by adding a suitable amount of triethanolamine As pointed out by v Hirsch & Peiffer the staining becomes more intense at the higher pH The lipid extraction was carried out with chloroform methanol 2 1 at 60 C for 1 hour

2 1% thionine in 0 5 tartaric acid according to Feyrter (1936 1942)

3 0 5 toluidine blue in water solution (26)

4 Acriflavine according to Hollander (16) to demonstrate sulphuric acid esters With this chemical compound as a fluorochrome the sulphuric acid esters can be detected in the fluorescence microscope through the light emitted which is of an orange yellow colour In addition the specimens prepared for fluorescence microscopy can be used for light microscopy after treatment with paradimethylaminobenzaldehyde which gives a bright orange or red reaction product Some specimens were extracted with chloroform and methanol before staining

5 Modified PAS (periodic acid Schiff) reaction for demonstration of cerebrosides (2 3 4) In order to eliminate unspecific PAS positive products the standard PAS procedure was preceded by successive blockades with chloramine T and 2 4-dinitrophenylhydrazine and by alkaline hydrolysis with NaOH Lipid

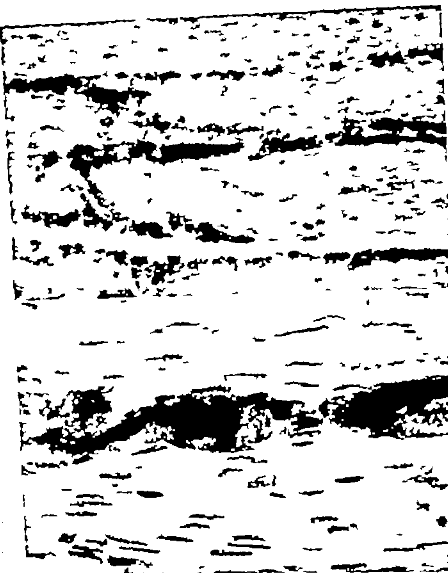


*Fig 1* Accumulation of metachromatic material in cells lying around blood vessels MLD case Frozen section Cresyl violet and acetic acid

*Fig 2* Peripheral nerve from one case of MLD The accumulated substance in these phagocytes is granular Frozen section Cresyl violet and acetic acid

Lipid extraction with chloroform and methanol was used to distinguish between polysaccharides and glycolipids reacting by the modified PAS procedure

6 Osmium tetroxide-α-naphthylamine method (OTAN) for demonstration of unsaturated phospholipids and unsaturated hydrophobic lipids (1 2)

7 OTAN preceded by alkaline hydrolysis with NaOH for sphingomyelin (2)

8 Scarlet red for hydrophobic lipids including cholesterol esters

B *Paraffin sections* The following staining methods were used on longitudinal sections 6–7 microns thick from the control nerves

1 Lowell fast blue-cresyl violet according to Kluver & Barrera (20)

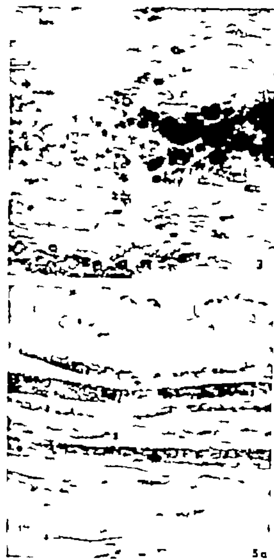
2 Palmgren's silver impregnating technique (35)



## RESULTS

### I *Peripheral neuropathy due to metachromatic leucodystrophy*

All cases of MLD verified post mortem displayed ample granular accumulations of metachromatic material in the nerves (Figs 1 and 2) These deposits appeared yellow or yellow brown with cresyl violet (Figs 1 and 2) dark brown to brown violet with toluidine blue (Fig 3) and grey brown to red brown with thionine Specimens treated with acriflavine showed an intense orange secondary fluorescence After



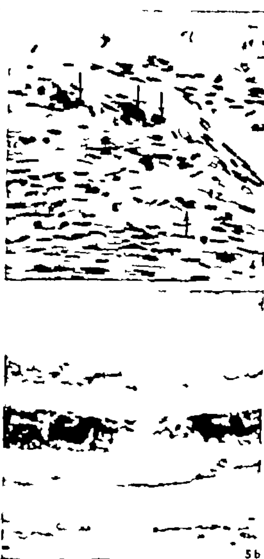


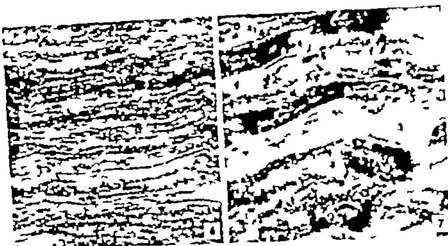
*Fig 3* The granular substance in the MLD nerves stains metachromatic with toluidine blue Frozen section

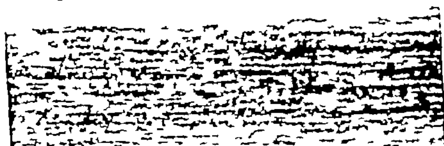
*Fig 4* Peripheral nerve from one case of MLD Arrows point to cells giving a positive reaction with Hollander's method Frozen section treated with paradimethylaminobenzaldelhyde

*Fig 5* Peripheral nerve from one case of diabetes mellitus with neuropathy Large amounts of metachromatic material can be seen in two fibres (*a*) The substance is granular and surrounds the nucleus (*b*) Frozen section Cresyl violet and acetic acid

subsequent treatment with paradimethylamino benzaldehyde the deposits were demonstrable in the light microscope by their orange to red colour (Fig 4) The amount of reacting material, however appeared to be smaller in the acriflavine treated sections than in the sections stained with cresyl violet One of the cases which gave a strong positive reaction with cresyl violet reacted negatively in the sections treated with acriflavine After lipid extraction with chloroform and methanol the deposits could no longer be demonstrated in sections stained with cresyl violet or acriflavine

The perivascular phagocytes in the nerves of the MLD cases appeared red after treatment with the modified PAS technique for cerebrosides (2 3 4) The staining reaction was not eliminated by the lipid extraction In the OTAN and Na OTAN preparations these cells appeared dark brown and the peripheral zone in the cytoplasm often had a bluish colour (due to Alcian blue used as a counter stain) In two of the MLD cases the cells appeared pale pink after staining with Scarlet red no sudanophilic products could be demonstrated in the other cases



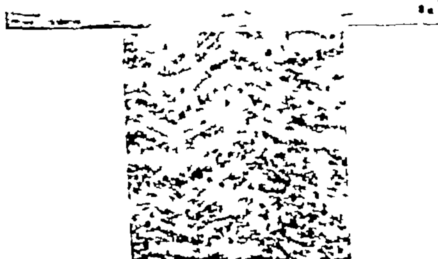
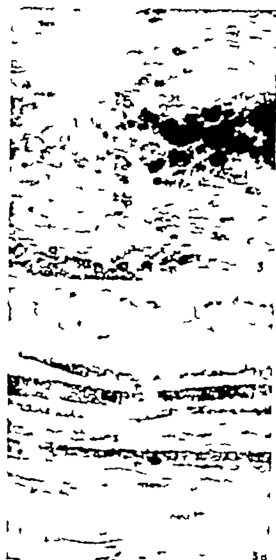



Fig. 6. Peripheral nerve from one case of uremia. Note the large amount of metachromatic substance. Frozen section. Cresyl violet and acetic acid.

Fig. 7. Peripheral nerve from one case of uremia. The metachromatic granules are mainly localized in Schwann cells. Frozen section. Cresyl violet and acetic acid.

Fig. 8. Metachromatic substance in peripheral nerve from controls (a and b). Frozen section. Cresyl violet and acetic acid.



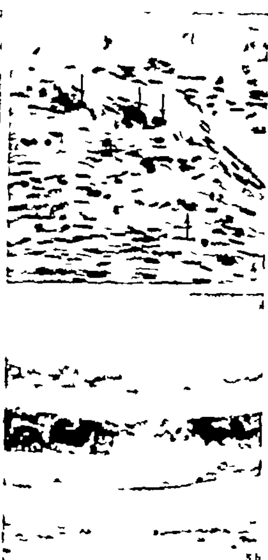


*Fig. 3.* The granular substance in the MLD nerves stains *metachromatic* with toluidine blue. Frozen section.

*Fig. 4.* Peripheral nerve from one case of MLD. Arrows point to cells giving a positive reaction with Hollander's method. Frozen section treated with paradimethylaminobenzaldehyde.

*Fig. 5.* Peripheral nerve from one case of diabetes mellitus with neuropathy. Large amounts of metachromatic material can be seen in two fibres (*a*). The substance is granular and surrounds the nucleus (*b*). Frozen section. Cresyl violet and acetic acid.

subsequent treatment with paradimethylaminobenzaldehyde, the deposits were demonstrable in the light microscope by their orange to red colour (Fig. 4). The amount of reacting material, however, appeared to be smaller in the acriflavine-treated sections than in the sections stained with cresyl violet. One of the cases which gave a strong positive reaction with cresyl violet reacted negatively in the sections treated with acriflavine. After lipid extraction with chloroform and methanol the deposits could no longer be demonstrated in sections stained with cresyl violet or acriflavine.

The perivascular phagocytes in the nerves of the MLD cases appeared red after treatment with the modified PAS technique for cerebrosides (2, 3, 4). The staining reaction was not eliminated by the lipid extraction. In the OTAN and Na-OTAN preparations these cells appeared dark brown and the peripheral zone in the cytoplasm often had a bluish colour (due to Alcian blue used as a counter stain). In two of the MLD cases the cells appeared pale pink after staining with Scarlet red; no sudanophilic products could be demonstrated in the other cases.

cleus In the newborn on the other hand the granules were uniformly distributed along the internode In one of the cases the granules were mainly located paranodally There was no accumulation of brown metachromatic products in perivascular phagocytes

The amount of brown metachromatic substance varied only slightly between different nerves i e nervus ischiadicus nervus femoralis and nervus suralis from the same individual On the other hand there were obvious quantitative variations in this respect between the different cases

## DISCUSSION

It has long been known that sulphatide is a normal chemical component of the myelin sheath (22) Histochemically sulphatide can be shown in adults in granular form both in individuals with structurally undamaged nerves and in certain pathological conditions (cf 6) The strong accumulation of metachromatic substance in peripheral nerves in children with MLD caused Thieffry & Lyon (34) at a time when the chemical character of the disease was not yet generally recognized (5 13 19) to recommend peripheral nerve biopsy as a diagnostic method in MLD

The method introduced by v Hirsch & Peiffer for establishing metachromasia in frozen sections of nerve tissue in MLD has been used for chromatographic identification of sulphatides extracted from tissues in MLD patients (13 19) The method has been considered as specific for sulphatides (2 33) Svennerholm has shown by quantitative chemical methods that the value for sulphatides in the sciatic nerve of an advanced case of MLD amounted to 80 % of the homoglycolipids (13 a) while the equivalent normal value for those nerves is 25

Considering the chemically proved normal occurrence of sulphatides in peripheral nerves in children and the diagnostic importance attached to the accumulation of metachromatic lipids (sulphatides) in peripheral nerves in MLD (14) it seems important to determine the degree to which and the condition in which histochemically demonstrable sulphatide granules may be present in peripheral nerves

The purpose of the present investigation was thus to sharpen the diagnostic criteria of MLD based on peripheral nerve biopsy By comparing verified cases of MLD with a group of cases of peripheral neuropathy with a cause other than MLD and with a group without clinical or morphological signs of peripheral nerve damage it should be possible to construct a basis for a more reliable differential-diagnostic judgment A recent series of investigations at our laboratory (21 32) has shown that peripheral neuropathy also occurs in inborn metabolic errors affecting the nervous system other than MLD (Krabbe s leucodystrophy infantile and juvenile amaurotic idiocy) and for this reason cases of these diseases have been included in the material

Our investigation has shown that sulphatides can be histochemically demonstrated in peripheral nerves in conditions other than MLD This means that differential-diagnostic difficulties may arise in judging peripheral nerve biopsies and that the mere presence of histochemically provable sulphatides is not a sure diagnostic criterion for MLD

The method most often used to establish sulphatides histochemically was introduced by v Hirsch & Peiffer (15) It is based on the fact that sulphatides with cresyl violet in an acidified solution give a brown metachromatic colour which does not occur after extraction with chloroform and methanol Instead of cresyl violet other aniline stains such as thionine and toluidine blue can be used but with these stains the metachromatic deposits are less distinctive Hollander (16 17) recently (1963 1964) described another method of demonstrating sulphatides in tissues based on the strong affinity of these compounds to acriflavine When we used this method on our MLD cases however the amount of positively reacting material seemed to be smaller than with the cresyl violet acetic acid method One of our chem-

One of the most characteristic features in the MLD nerves was that the granular deposits were always localized to phagocytes that, singly or in large clusters surrounded vasa nervorum or lay just under the perineurium The occurrence of these phagocytes however, varied widely between the different cases Metachromatic granules were also seen in myelin sheaths and in Schwann cells and often as free-lying granules between the fibres

II *Peripheral neuropathy not due to metachromatic leucodystrophy*

*Globoid cell leucodystrophy* In globoid cell leucodystrophy there is an accumulation of a granular substance in Schwann cells and in endoneurial phagocytes which gives a positive reaction with Adams method (2) for cerebrosides As has been pointed out previously these accumulations do not give any brown metachromatic colour reaction with cresyl violet and acetic acid (31) Single brown violet granules do however occur in a few fibres The stainability of these granules disappeared after lipid extraction with chloroform and methanol

*Infantile amaurotic idiocy* Sections from spinal nerve roots displayed brown violet granules mainly accumulated round the nodes of Ranvier Single similar granules also appeared at random along the internode Remarkably enough these metachromatic products were not eliminated with the lipid extraction though the colour shifted to red violet

*Juvenile amaurotic idiocy* In all nerves examined from cases of juvenile amaurotic idiocy there were dark to brown violet metachromatic inclusions in Schwann cells These were however very sparse Single yellow brown granules were also observed lying free between the nerve fibres in the endoneurium A massive accumulation of such granules in typical phagocytes was not observed

*Diabetes mellitus* The amount of brown metachromatic products varied considerably in specimens from different diabetic cases stained with cresyl violet and acetic acid Severely damaged nerves contained only a small amount of brown metachromatic substance while better preserved specimens revealed moderate to large amounts of metachromatic granules in Schwann cells often gathered in clusters round the nucleus (Fig 5) These granules appeared dark brown to brown violet Sometimes similar granules were seen lying free between the fibres in the endoneurium There was no accumulation in phagocytes In all cases the amount of brown metachromatic substance was considerably smaller than in the MLD cases

*Cancer and uremia cases* In all cancer and uremia cases examined there was plenty of brown metachromatic substance with the same localization as in the diabetic cases (Figs 6 and 7) Compared with the latter however the amount of substance was considerably greater and the colour variations were more marked from yellow brown to brown violet The cases which showed signs of active break down of the myelin sheaths contained particularly large amounts of brown metachromatic products and in that respect resembled MLD There was no massive accumulation of metachromatic substance in phagocytes in these cases either

III *Nerves without morphological signs of pathological changes*

All nerves from the control cases stained by v Hirsch & Peiffer s method (15) presented dark brown to brown violet metachromatic granules (Fig 8) In the specimens stained with thionine the granules appeared grey brown and after staining with toluidine blue they were blue violet Both of Hollander s methods gave negative results The chloroform methanol extraction totally eliminated the metachromatic staining of these granules In single nerves the granules were gathered in larger clusters Sometimes the substance was more diffusely spread and could not be distinguished as single granules

The brown metachromatic granules seemed to be located to the cytoplasm of the Schwann cells In nerves from adults such granules were often gathered round the Schwann cell s nu

suspected metachromatic leucodystrophy peripheral nerves were examined from individuals belonging to three different categories viz cases of metachromatic leucodystrophy (MLD) verified chemically and post mortem cases of other diseases afflicting the peripheral nerves and cases with no clinical or morphological evidence of neurological disease The method recommended for the diagnostic establishment of sulphatides is that described by v Hirsch & Peiffer with cresyl violet and acetic acid applied to frozen sections Using this method the sulphatides appear as brown metachromatic granular substances which are completely dissolved by chloroform and methanol The most important results of the present investigation can be summarized as follows

1 In advanced cases of MLD there is a copious accumulation of a yellow brown metachromatic granular substance localized partly to Schwann cells partly to perivascular or subperineurial phagocytes In addition there are advanced axon and myelin lesions

2 In peripheral neuropathies due to causes other than MLD and in normal peripheral nerves there are also varying amounts of brown metachromatic granules localized to nerve fibres However no massive accumulation occurs in perivascular or subperineural phagocytes

For the present a reliable diagnosis of MLD by peripheral nerve biopsy requires the demonstration of sulphatide accumulation in typical phagocytes In those cases in which the brown metachromatic substance can only be traced to nerve fibres and Schwann cells no reliable diagnosis of MLD can be made but neither can the possibility of an early stage of MLD be excluded

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cally and histochemically confirmed cases of MLD which showed a strong positive meta chromatic reaction with cresyl violet and acetic acid showed a negative reaction with Hollander s method For these reasons, we primarily recommend v Hirsch and Peiffer s technique for the diagnosis of MLD

*The most characteristic and constant phenomenon* in the nerves of the MLD cases was the accumulation of a yellow brown lipid extractable substance in frozen sections stained by v Hirsch & Peiffer s method This substance occured both *in the nerve fibres and in* the cytoplasm of perivascular or subperineurial phagocytes (Figs 1 and 2) Recently the presence of sulphatides in the Schwann cell s cytoplasm in MLD cases was elegantly demonstrated with the teasing technique (7) and by electron microscopy (35) The accumula*tion of sulphatides in endoneurial phagocytes* in MLD cases has previously been pointed out especially by Hagberg *et al* (14) and by Norman *et al* (23) In addition to the accumulation of sulphatides in Schwann cells and in macrophages lesions that are partly of a segmental character occur in the myelin sheaths (7 11 35)

In all of the normal peripheral nerves examined by us from the foetal stage onwards brown metachromatic lipidextractable granules were present in the nerve fibres Granules with a corresponding localization and stainability have previously been observed in human peripheral nerves (6 27 28 29 30) These Reich granules are constantly present in adult cases but they have not been observed before in peripheral nerves of humans under four years of age (cf 6) The differential diagnosis between normal peripheral nerves containing brown metachromatic granules in Schwann cells and advanced cases of MLD can hardly cause any difficulty since the latter constantly show up take *of sulphatides in endoneurial* phagocytes besides showing signs of axon and myelin sheath changes On the other hand diagnostic difficulties may possibly arise in early cases of MLD where there is no admission into the *phagocytes and where axon and myelin sheath* changes may be difficult to prove

Even in cases of globoid cell leucodystrophy and cases of infantile and juvenile amaurotic idiocy small amounts of brown metachromatic granules may be seen in the peripheral nerve with the same localization as in normal peripheral nerves Since considerable damage t myelin sheaths and axons of the nerves ma occur in these conditions (21 31 32) the differential diagnosis is made more difficult with regard to MLD As histochemical methods do *not allow reliable quantitative estimates of the* amounts of sulphatide present in the nerves it is necessary to look for structural differences in the mode of accumulation of sulphatide granules in MLD and other conditions The accumulation of sulphatide in the phagocytes in the MLD cases which has not been observed *in other conditions represents* such a difference

Our material consists of nerves from advanced cases of metabolic and degenerative diseases in children involving the nervous system It should be possible to make a sure diagnosis of MLD by peripheral nerve biopsy *in the advanced cases but in early stages* of the disease considerable difficulties may arise with regard to other types of peripheral neuropathy In particular it seems likely that the early cases of MLD contain more discrete amounts of histochemically establishable sulphatide than the more advanced cases and that *therefore* the accumulation in phagocytes is less obvious When this diagnostic criterion *is not available the determination as against* early stages of other peripheral neuropathies may become difficult since the amount of histochemically provable sulphatide in a current myelin breakdown seems to be particularly pronounced as suggested by our observations on uremia and cancer cases

## SUMMARY

In order to increase the certainty of the diagnosis from peripheral nerve *biopsy in cases of*

*Acta Pædiat Scand 58 25–32 1969*

# THE LONG TERM PROGNOSIS OF NON OBSTRUCTIVE URINARY TRACT INFECTION IN INFANCY AND CHILDHOOD AFTER THE ADVENT OF SULPHONAMIDES

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Significant bacteriuria has been found among schoolgirls in 1% (10) and among an out patient paediatric clientele in 1 6 (15) Autopsy materials show pyelonephritis among children in between 2 (1937) (3) and 2 6 % (1962) (17) In autopsy materials pyelonephritis has been found among adults in 2 3 and 5 2 in women and 2 6 and 7 5 % in men (8 9) It is not known whether there is a relationship between urinary tract infection in childhood and chronic pyelonephritis among adults The facts that pyelonephritis in lower age-groups often is asymptomatic and that a minimum of 1 out of 4 cases of urinary tract infection in children recur in spite of treatment (1 4 14) suggest such a relationship

In reinvestigations including obstructive and non-obstructive cases of urinary tract infections in children (2 11 12 16 18 19 21) it has been found that in spite of being free of symptoms an appreciative minority with early recurrence develop slight radiological renal changes and in some cases serious damage Early recurrence seems to increase the risk of chronic renal disease (21) A close follow up of all cases has therefore been recommended

The present-day increased vigilance against obstructive urinary tract infection leads to early detection and surgical treatment of lower urinary malformations This might have changed the prognosis in this group considerably and it therefore seems meaningful to separate obstructive and non-obstructive cases in reinvestigations of urinary tract infection in children

This study was made possible through a grant from Svenska Livförsäkringsbolags Nämnd för Medicinsk Forskning

## MATERIAL

One hundred and twenty-eight cases were hospitalized for urinary tract infection in the university clinic for paediatrics at Crown Princess Lovisa's Children's Hospital in Stockholm during the years 1940–1949 They were all seen by Professor A Lichtenstein, former head of this clinic which suggests uniform criteria A high frequency of radiological investigation indicates vigilance against obstructive disease Out of these cases 18 showed malformations 16 with obstruction Two cases had tuberculosis of the urinary tract and 1 had diabetes mellitus These 21 cases were excluded from the reinvestigation

The 107 remaining cases of non-obstructive urinary tract infection filled the criteria of urinary tract infection according to Steele *et al* (18) i e 2 criteria out of (1) positive culture (2) significant leucocyturia and (3) marked symptoms (Table 1) Sex-distribution and age of debuts is given in Fig 1 The distribution in different ages is the typical one and the material can therefore be regarded as representative The patients had all been treated with sulphonamide for around one week to a normal urinary sediment a normal body temperature and until they were free of symptoms One or more controls had been done after therapy in the out patient department The urinary examinations had been done on catheter samples (Table 1) In 67 cases the infecting organism was determined by culture (Table 2) Sixty-eight cases 16 under 6 months of age had been examined by i v pyelography Nephrectomy had been done in 3 cases of hydronephrosis

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# THE LONG TERM PROGNOSIS OF NON OBSTRUCTIVE URINARY TRACT INFECTION IN INFANCY AND CHILDHOOD AFTER THE ADVENT OF SULPHONAMIDES

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Significant bacteriuria has been found among schoolgirls in 1 (10) and among an out patient paediatric clientele in 1 6 / (13) Autopsy materials show pyelonephritis among children in between 2% (1937) (3) and 2 6 (1962) (17) In autopsy materials pyelonephritis has been found among adults in 2 3 and 5 2% in women and 2 6 and 7 5 in men (8 9) It is not known whether there is a relationship between urinary tract infection in childhood and chronic pyelonephritis among adults The facts that pyelonephritis in lower age-groups often is asymptomatic and that a minimum of 1 out of 4 cases of urinary tract infection in children recur in spite of treatment (1 4 14) suggest such a relationship

In reinvestigations including obstructive and non-obstructive cases of urinary tract infections in children (2 11 12 16 18 19 21) it has been found that in spite of being free of symptoms an appreciative minority with early recurrence develop slight radiological renal changes and in some cases serious damage Early recurrence seems to increase the risk of chronic renal disease (21) A close follow up of all cases has therefore been recommended

The present-day increased vigilance against obstructive urinary tract infection leads to early detection and surgical treatment of lower urinary malformations This might have changed the prognosis in this group considerably and it therefore seems meaningful to separate obstructive and non obstructive cases in reinvestigations of urinary tract infection in children

This study was made possible through a grant from Svenska Livförsäkringsbolags Nämnd för Medicinsk Forskning

## MATERIAL

One hundred and twenty-eight cases were hospitalized for urinary tract infection in the university clinic for paediatrics at Crown Princess Lovisa's Children's Hospital in Stockholm during the years 1940–1949 They were all seen by Professor A Lichtenstein, former head of this clinic which suggests uniform criteria A high frequency of radiological investigation indicates vigilance against obstructive disease Out of these cases 18 showed malformations 16 with obstruction Two cases had tuberculosis of the urinary tract and 1 had diabetes mellitus These 21 cases were excluded from the reinvestigation

The 107 remaining cases of non-obstructive urinary tract infection filled the criteria of urinary tract infection according to Steele *et al* (18) i e 2 criteria out of (1) positive culture (2) significant leucocyturia and (3) marked symptoms (Table 1) Sex-distribution and age of debute is given in Fig 1 The distribution at different ages is the typical one and the material can therefore be regarded as representative The patients had all been treated with sulphonamide for around one week to a normal urinary sediment a normal body temperature and until they were free of symptoms One or more controls had been done after therapy in the out patient department The urinary examinations had been done on catheter samples (Table 1) In 67 cases the infecting organism was determined by culture (Table 2) Sixty-eight cases 16 under 6 months of age had been examined by i v pyelography Nephrectomy had been done in 3 cases of hydronephrosis

Table 1 *Symptoms and laboratory findings in the 107 cases of the primary material*

| | Cases |
|---|---|
| *Symptoms* | |
| Temperature 38 | 92 |
| Pollacuria | 37 |
| Burning micturition | 33 |
| Costovertebral pain or tenderness | 13 |
| Secondary enuresis | 13 |
| *Laboratory findings* | |
| Pyuria | 102 |
| Sedimentation rate >20 mm | 78 |
| Organism cultured | 67 |
| Leucocytosis >12000 | 58 |
| Proteinuria | 54 |
| Haematuria | 12 |
| Blood pressure >90 diast | 0 |

The 107 cases were divided according to Steele *et al* (18) into

1 Urinary infection (whole material)
2 Probable acute pyelonephritis i e 2 criteria out of (*a*) costovertebral pain or tenderness (*b*) body temperature above 38 C or white blood corpuscles in blood above 12 000 or sedimentation rate above 20 mm and (*c*) moderate changes of calyces on i v pyelography
3 Acute pyelonephritis i e 1 criterium out of (*a*) pathological anatomical diagnosis (*b*) marked changes on i v pyelography and severe symptoms and signs clinically (Table 3)

## METHODS

The reinvestigation consisted of uptake of a case history in conference with all the surviving patients and if possible the parents and grandparents as well Genetic factors symptoms hospitalizations visits to doctors and pregnancies were dealt with General condition blood pressure after bed rest haemoglobin sedimentation rate specific true serum-creatinine protein in urine and leucocytes in uncentrifugated urine were estimated Maximum urinary concentration capacity under pitressine load was determined At least one quantitative culture on voided urine was done An i v pyelography was done in 52 of the 73 surviving cases Micturition urethrocystography was done in 9 cases because of abnormality indicated by the re investigation

Criteria for recurrence during the observation period were the same as those of the primary material For urinary tract infection at the time of the reinvestigation the criteria were above 100 000 bacteria per ml of urine (6) and leucocytes above 25/mm in men and 50/mm in women In addition to those of actual infection the criteria for actual parenchymal infection were body temperature above 38 C sedimentation rate above 20 mm and a transitory lowering of the maximum concentration capacity (20)

As normal results were regarded diastolic blood pressure below 90 mm Hg haemoglobin for men above 13 4 g and for women above 11 9 g sedimentation rate below 20 mm h true serum creatinine for men below 1 2 mg and for women below 1 0 mg% leucocytes in uncentrifugated urine below 25/mm for men and below 50/mm for women maximum concentration capacity under pitressine load above 800 mOsmol/l and quantitative urine culture below 10 000–100 000 bacteria per ml urine

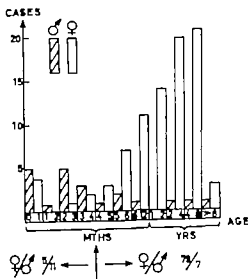



*Fig 1* Sex distribution and age at first noted infection

## RESULTS

Out of the primary 107 cases 2 had died during the debute infection and three had died later according to the reinvestigation giving a minimum total mortality of 5% All dead were infants at the time of the debute and they were all dead within 3 years of their first diagnosed infection No autopsies had been done and the cause of death is unknown Tentative diagnosis from available data would be whooping cough at one year of age in one case mucoviscidosis

Table 2 *Organisms found in the first noted infection of the 107 cases*

| Bacteria | Cases |
|---|---|
| E Coli | 57 |
| Enterococcus | 7 |
| Staphylococcus aureus | 2 |
| Proteus | 1 |

in one case and multiple malformations in one case

Seventy six of the 105 primarily surviving cases were reinvestigated. In 15 of the remaining cases no contact could be established: 11 were unwilling and 3 had emigrated. Unwillingness in the cases who were later reinvestigated did not seem to be a guarantee of health.

Progressive parenchymatous reductions of the kidneys at the time of the reinvestigation were found upon X-ray in 11 cases, all women. Twenty eight cases had late recurrence, all women. Symptoms from the urinary tract at the time of the reinvestigation were claimed in 22 cases, all women. In view of the lack of positive findings in the 18 reinvestigated men, the further presentation is concentrated on the 58 reexamined women (Table 4).

After the 15–25 years observation time 11 of the 58 women or 19% showed progressive upper urinary tract disease according to par-

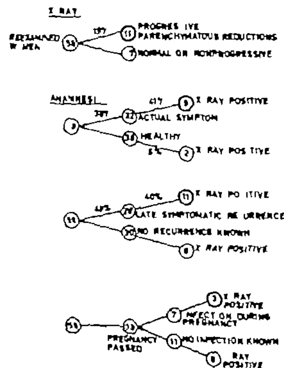



*Fig. 2.* Results according to radiological examination in correlation to symptoms as costovertebral pain, fever with pollacuria, given in conference with the patient at the time of the reinvestigation.




*Fig. 3.* Results according to radiological examination in correlation to early recurrence, i.e. recurrence in maximum one year's time from the primary hospitalization.

enchymatous reductions on radiological examination. In these 11 cases all had symptomatic recurrences during the last 5 years: 6 with fever above 38° C, lumbo sacral pains and burning micturition. Three of the 11 cases showed actual parenchymatous infection at the time of the reinvestigation. Only 2 of the 11 cases were under medical care because of chronic pyelonephritis before the time of the reinvestigation. The clinical signs were as shown in Table 5. Chronic urinary infection in the family was known in 3 of the 11 cases.

Fig. 2 shows the results according to radiological examination in correlation to symptoms given in conference with the patient and her parents. Fig. 3 shows the results according to radiological examination correlated to early recurrence, i.e. recurrence in maximum one year's time from the primary hospitalization.

## DISCUSSION

The material must be considered as selected, consisting only of hospitalized cases. However hospitalization at the time of debute seemed to us necessary when the primary clinical and radiological examination was important. Fever above 38° C was present in 92 of the 107 cases, suggesting a high frequency of primary acute pyelonephritis. From Table 3 it is evident that the percentage of reinvestigated cases is evenly distributed among the different primary classifications with an under-representation of "possible acute pyelonephritis". This fact in combination with the fact that all persons reinvestigated could not agree to radiological investigation indicates that the results show minimum figures for found cases of chronic pyelonephritis.

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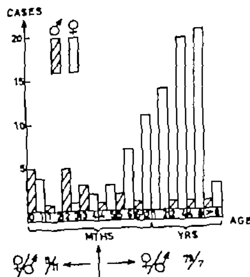



*Fig 1* Sex distribution and age at first noted infection

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| Proteus | 1 |



*Fig. 4.* Retrograde pyelography at 5 months of age. Left side. Upper calyces normal, middle and lower calyces probably pathological. Right kidney normal. Both kidneys grossly of the same size.

slight tenderness over the left kidney region. There was no proteinuria, leucocyturia or bacteriuria. Sedimentation rate, haemoglobin and differential count on white blood corpuscles were normal. Serum "true creatinine was 0.8 mg%" and maximum concentration capacity after pitressine load 960 mOsmol/l or normal. I.v. pyelogram was pathological (Fig. 5). Six months after the reinvestigation the patient was suffering from right abdominal pain and because of the recent re-investigation the paediatric clinic was contacted. The patient was referred to a urological adult unit. Cultures were negative. A seriously pathological function of the left kidney was found on split function test. Creatinine-clearance right kidney 63 ml/min and left 10.13 ml/min. PAH-clearance right kidney 858.83 and left 63.94 ml/min. Regular control at the urological unit was instituted at 17 years of age with the diagnosis of pyelonephritis chronica.

All former patients could not possibly agree to a reinvestigation including detailed functional tests and the screening was progressive radiological changes as main criterium (5, 15). The figure of 19 % of the women showing radiologically evident progressive changes must be seen as a minimum figure of chronic or recurrent pyelonephritis in view of the fact that 38 % were complaining of actual symptoms suggestive of urinary disease and 48 % having had a late recurrence (Fig. 2). It seems evident from the correlation in Figs. 2 and 3 that a thorough case history is a good help in screening out possible radiologically progressive renal disease in young women with urinary infections. The fact of an earlier infection should lead to a study of the original records usually hidden in the paediatric files. According [illegible] re-investigation actual symptoms [illegible] patient increased the incidence [illegible]

Table 3 *Clinical reclassification of the primary material (18) i e reevaluation from data in the original papers*

| Reclassification | Cases | Sex M | Sex F | Reinvestigated No | Reinvestigated % |
|---|---|---|---|---|---|
| Urinary infection (total) | 107 | 21 | 86 | 18 M, 58 F } 76 | 76 M, 70 F } 71 |
| Possible acute pyelonephritis | 27 | 5 | 22 | 15 | 56 |
| Acute pyelonephritis | 8 | 3 | 5 | 6 | 75 |
| Recurrent or chronic infection | 51 | 6 | 45 | 39 | 75 |
| Febrile >38 C | 92 | 20 | 72 | 64 | 70 |

Fifty-one cases in the primary material of 107 cases showed early recurrence, which should be compared to the present day recurrence of 1 out of 3–4 (1 4 14) The treatment given to the patients in the 1940s does not however seem to us to be principally different from the present-day customary one This difference might depend on the fact that all present cases were hospitalized and represent more serious infections and that the treatment in the follow up series mentioned was extended over a minimum of 10 days, while in the present material the sulpha treatment was given for 6–7 days only

The sex-distribution in Fig 1 and the good prognosis found in the low number of boys is in agreement with De Luca s statement (4) that non obstructive infections in boys give symptoms earlier and show a better prognosis than in girls

The diagnosis of chronic pyelonephritis in the age-group of the reinvestigated cases (15–30 years) when pyelonephritis is known to be mainly asymptomatic, is evidently the greatest difficulty in an investigation of this kind In order to demonstrate this the following case is reported

*Case report*

G T girl born Jan 21 1949 548/49 Healthy mother with normal second delivery Hypogalactia and cowmilk formula was given from 1 month of age Weight increase 700 g/month At 3 months of age sudden illness with fever 40 9 C gray coloured skin and dyspnea Sulpha was given for a couple of days by a practitioner for suspected pneumonia There was proteinuria Stools were loose and frequent After 14 days 38 4 C cough and irritable Admitted Pharyngeal culture and chest X ray negative Pale haemoglobin 7 9 g% Haematocrite 24 sedimentation rate 70 mm/h Proteinuria pyuria and gram positive cocci in catheterized urine Urinary cultures 3 times before treatment showed Enterococci and Aerobacter aerogines Penicillin and sulpha was given for 6 days Temperature was normalized in two days Urinary sediment became clear The weight dropped 5% initially and stayed low for 14 days On the 10th day there was fever 38 6 C and a culture on the 12th day showed Aerobacter aerogines On the 20th day a negative urinary culture was achieved without treatment There was a suspicion of a right sided hydronephrosis on i v pyelography and the patient was transferred to the surgical unit

The suspicion of hydronephrosis was abondoned after a normal retrograde pyelography (Fig 4) Repeated cystoscopies were normal During the next 9 months the patient was hospitalized 5 times for febrile urinary tract infections In every instance the clinical signs were fever initial weight loss pyuria proteinuria bacteriuria (E Coli) and anemia The treatment was sulpha for 5 to 9 days and at one recurrence streptomycin was given for 10 days after growth of sulpha resistant E Coli Negative cultures well after treatment were noted after recurrences The patient was asked to come back for check ups in the open ward and did so twice up to 14 months of age or 6 weeks after last noted recurrence

Reinvestigation 15–17 years of age The patient and her father were unwilling to cooperate in a reinvestigation but agreed if only simple tests were to be made The father could report that the girl had cystitis at 3–4 years of age but after that no trouble and was regarded as healthy There was no family history of urinary disease The girl appeared healthy blood pressure was 115–120/80 mm Hg There was

Table 4 *Radiological findings in the 76 reinvestigated cases*

| Sex | Normal | Deformed calyces | Parenchymal reduction |
|---|---|---|---|
| *First infection* | | | |
| M | 3 | 4 | 2 (operated) |
| F | 26 | 14 | 1 |
| Total | 29 | 18 | 3 |
| *Reinvestigation* | | | |
| M | 6 | 2 | 0 |
| F | 33 | 12 | 11 |
| Total | 39 | 14 | 11 |

All 11 women had been investigated radiologically during the first hospitalization

Two of the cases with later confirmed chronic pyelonephritis suffered immediately after the reinvestigation from a symptomatic recurrence in spite of many years absence of any symptoms. We have later observed in 4 cases of pyelonephritis in children how significant bacteriuria also symptomatic could be achieved during the maximal dehydration of a pitressine load. In one case J H 879/67 a 3 years old girl with recurrent febrile urinary tract infections known since 6 months of age and with normal radiological investigation 7 cultures performed because of pollacuria 3 months after the 10 days initial nalidixic acid treatment were negative while significant bacteriuria could repeatedly be achieved during maximum dehydration under a pitressine load. This could mean that in the 2 mentioned cases of the reinvestigation recurrence was triggered by the dehydration. Such a dehydration could be a way of checking the effect of the therapy given as well as serving as an additional test for chronically recurrent urinary tract infections.

## SUMMARY

One hundred and seven cases of urinary tract infection in children hospitalized during the years *1940–1949* were reinvestigated with an observation time ranging from 15 to 24 years. Primary and late mortality was low. Non obstructive urinary infections in boys show a good prognosis concerning recurrence as well as progressive renal disease.

Non-obstructive urinary tract infection in girls seems to be a potentially serious disease where recurrence developed in half the cases in spite of the initial sulpha treatment given. A minimum of 19 % of the girls showed progressive renal disease according to radiological examinations. Early recurrence according to the primary report, increased the risk of progressive renal disease from 4 to 30 %. In 40 % of the girls with late symptomatic recurrence progressive renal disease could be diagnosed at about 20 years of age.

The reinvestigation shows that in certain individuals the child's susceptability to recurrent urinary tract infection is retained even in the adult in whom it manifests itself as acute pyelonephritis. It is of special interest to note that between one half to one third of those who had been pregnant had suffered from urinary tract infection of pregnancy.

At about 20 years observation time negative results of single determination of blood pressure, sedimentation rate, haemoglobin, serum creatinine, protein or leucocytes or bacteria in the urine and maximal urinary concentration capacity does not seem to guarantee the absence of chronic or recurrent pyelonephritis. A thorough case history including a paediatric history is recommended in dealing with urinary tract infections in children and young adults.

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Fig 5 Intravenous urography at 16 years of age Left kidney small calyces very irregular and distorted without papillae Right kidney large with thick parenchyma and normal calyces (A small calculus in one of the upper calices of the right side is here obscured by the contrast medium)

Table 5 *Clinical signs at reinvestigation of the 58 women*

| Clinical signs | Progressive radiologically (11 cases) | Non progressive radiologically (47 cases) |
|---|---|---|
| Leucocyturia | 4 | 2 |
| Proteinuria | 4 | 3 |
| Bacteriuria | 3 | 2 |
| Lowered maximum concentration capacity (< 800 mOsmol/l) | 3 | 2 |
| True creatinine pathological (> 1 0 mg ) | 2 | 7 |
| Sedimentation rate pathological | 0 | 0 |
| Blood pressure pathological | 0 | 4 |
| Anemia | 2 | 8 |

renal changes 7-fold late symptomatic recurrence increased the incidence to 40 and the fact of an early recurrence mentioned in the records gave positive radiological findings with a 7 fold increase The time of follow up did not seem to be correlated to the number of patients with progressive renal disease in this investigation where the time of follow up varied in between 15 and 24 years

The fact of 7 cases with recurrence during pregnancy among the 18 who had been pregnant (Fig 2) should be compared to 5 with bacteriuria during pregnancy in a normal material (7) Of the 7 women 3 had progressive renal changes and one of these had given birth to a stillborn premature

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# ASSESSMENT FOR ADOPTION

## *A Follow up Study*

R S ILLINGWORTH

*From the Department of Child Health University of Sheffield Sheffield England*

It is almost a truism to say that unless one follows up one's patients one has little chance of learning from one's mistakes When attempting developmental assessment in infancy one cannot hope to learn the difficulties and dangers of assessment and the limitations of one's methods unless one follows up those infants which one has assessed In the case of assessment for suitability for adoption it is not easy to ensure that all babies assessed are followed up to school age Thanks to the excellent co-operation of Nottingham County Council together with the desire of its Children's Department that assessments should be as accurate as possible and that their degree of accuracy should be determined all babies assessed by me are examined by psychologists when they are at school It is thus possible to compare the I Q scores at school at the age of 6 or 7 years with the grading allocated in infancy This report is an initial study of the first 156 infants assessed by me in the first year of life and 36 different children seen in the second year and reexamined by others at the age of six or seven years

Opinions differ as to whether developmental assessment in infancy has any predictive value at all I have reviewed the literature concerning this elsewhere (2) A report from the Child Adoption Research Committee in New York declared that It is practically a truism that performance in a baby test alone is no accurate indication of the level of intellectual development of an individual in the future (1951) However all retarded children were excluded from the study no infant having a test score below 90 Wittenborn (3) attempted to determine the value of the assessment of infants in Gesell's clinic for the purpose of adoption by means of a follow up examination with Binet tests at the age of 5 years or more and concluded that We find no means of refuting a hypothesis that the infant examination has no useful predictive value Although we cannot prove that the hypothesis of no predictive validity is true it describes our data But Wittenborn had already excluded all defective children before examining infants used in this study He then selected a group of those already selected children including highly selected infants of members of the Yale University staff and then reassessed 114 of the group of 310 at 5 or later not examining the remaining 196 The reader is invited to believe that those not followed up would be the same as those followed up

## MATERIAL AND METHOD

The babies were unselected ones referred by the Nottingham County Council for assessment of suitability for adoption There were no babies with mongolism cretinism or hydrocephalus They were mostly seen at the age of six months in the first place and again at 10 months if there were doubt about the baby being normal at the first examination The total number followed was 192 Of these 156 were assessed in the *first* year while 36 other children were first assessed in the second year The majority of the babies including nearly all those seen in the early part of the study were brought from two

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Stockholm K
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*Key words* Urinary tract infection infancy and childhood long term prognosis

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# ASSESSMENT FOR ADOPTION

## *A Follow up Study*

R S ILLINGWORTH

*From the Department of Child Health University of Sheffield Sheffield England*

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children thought in the first year to be average or possibly better proved to have an I Q score at school of below 80 (1 8 per cent) whereas 3 of 46 children thought to be doubtful or retarded had an I Q score of over 120

Many of the infants came from a thoroughly unsatisfactory background and 21 had a certified mentally defective mother or father (or in one case both) Table 4 shows the separate analysis of these cases (which are included in the other tables) It is particularly notable that the mean I Q score at school age was 100 1 It will be seen that 12 had an I Q score of over 110 One had an I Q score of 125 This indicates how wrong it would be to reject a child for adoption merely because a parent is mentally defective Each child should be assessed on his merits

## DISCUSSION

Developmental predictions never will be accurate for there are so many variables which may affect the child's subsequent progress They include the nature of the home the degree of stimulation given by the parents the sort of toys provided the amount of time devoted to

Table 3 *Number of children who at school had an I Q score of below 80 or over 120 in relation to original grading*

| | | I Q score at school | | | |
|---|---|---|---|---|---|
| | | Below 80 | | Above 120 | |
| | Total | Number | % | Number | % |
| *Grading in first year* | | | | | |
| 1 or (Average or possibly better) | 110 | | 1 8 | 18 | 16 3 |
| 3 or 4 (Doubtful or retarded) | 46 | 7 | 15 | 3 | 6 6 |
| *Grading in second year* | | | | | |
| 1 or | 16 | 0 | — | 1 | — |
| 3 or 4 | 70 | 4 | — | 2 | — |

Table 4 *Infants of a mentally defective parent*
I Q score at school in relation to grading in infancy

| I Q score at school | Grading in infancy | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Total |
| Below 90 | — | 1 | 2 | 2 | 5 |
| 90–99 | — | 1 | 2 | 1 | 4 |
| 100–109 | — | 2 | 1 | — | 3 |
| 110 or over | 3 | 1 | 5 | — | 9 |
| Total | 3 | 5 | 10 | 3 | 21 |
| Mean I Q score | 116 3 | 101 0 | 100 0 | 87 7 | 100 1 |

reading to them the degree of happiness and security experienced and at school the quality of the teaching and the whole school environment Physical illness may retard a child Some may acquire lead poisoning and others may have degenerative diseases of the nervous system Some develop meningitis or a head injury with sequelae I have discussed the retarding factors and factors which improve performance in another place The additional difficulty in this series was a major one—the fact that the majority of babies had suffered emotional deprivation and so were retarded as a result One could only guess how much of a child's apparent backwardness was due to this factor

Nevertheless the results do show that the developmental examination at the age of six months was of value in assessing suitability for adoption for those who proved to be backward later were in nearly all cases picked out at the age of six months It is far more important for the would be adopting parents that one should be able to pick out mental subnormality and cerebral palsy than that one should be able to detect mental superiority The difficulty of detecting mental superiority in early infancy is wellknown

## SUMMARY

A total of 156 babies were assessed for suitability for adoption in the first year almost all of them at the age of six months and a further

Table 1 *Mean I Q at school in relation to grading in infancy*

| | Total | Mean I Q at school |
|---|---|---|
| *Grading in first year* | | |
| 1 (Possibly above average) | 41 | 109 1 |
| 2 (Average) | 69 | 107 3 |
| 3 (Doubtful) | 36 | 99 8 |
| 4 (Retarded) | 10 | 84 1 |
| 1 and 2 (Average or possibly better) | 110 | 108 0 |
| 3 and 4 (Doubtful or retarded) | 46 | 95 0 |
| *Grading in second year* | | |
| 1 and 2 | 16 | 107 5 |
| 3 | 15 | 97 1 |
| 4 | 5 | 88 0 |
| 1 and 2 | 16 | 107 5 |
| 3 and 4 | 20 | 95 0 |

long stay homes for children in care This is important because they had suffered emotional deprivation and were retarded on that account This made the assessment particularly difficult In the latter part of the study the Homes were no longer used for the purpose the current practice being to place the babies at the age of 10 days in a foster home with foster parents who wish to adopt

The babies were brought into the consulting room fully dressed and the initial part of the examination was made with the baby on the mother s knee The developmental and general history were then taken and the rest of the examination was completed after the child had been stripped The developmental part of the examination was modified from Gesell as described elsewhere The physical examination was the usual full one including examination of the hip for congenital subluxation the back for a congenital dermal sinus the maximum head circumference a test of hearing and examination of the urine for phenylpyruvic acid

On completion of the examination the child was placed in one of four grades and the figure was written into the notes The grades were as follows

*Grade 1* Possibly above average
*Grade 2* Average
*Grade 3* Doubtful possibly retarded
*Grade 4* Retarded

At school age mainly at the age of six to seven the children were examined by psychologists under the Nottingham County Council and elsewhere so that the I Q score could be obtained The tests used were mainly the Terman & Merrill and Stanford Binet The grade allotted by me was not known to the psychologists

The follow up was virtually complete only two or three being lost to the study

## RESULTS

Table 1 shows the mean I Q score at school age in relation to the grading in infancy It will be seen that the mean I Q of children placed in the first year in grade 1 (Possibly above average) was 109 1 that of those in grade 2 was 107 3 of those in grade 3 was 99 8 and that of those placed in grade 4 was 84 1 Combining these into pairs those thought in the first year to be average or possibly better had a mean I Q score of 108 and those thought to be doubtful or retarded had a mean score of 95 0 It is interesting to note that the figures for those children assessed only in the second year were very similar

Table 2 shows the gradings and results in more detail and Table 3 is a summary of Table 2 It is clear that the tendency was to underestimate potential rather than to over estimate it This was to be expected because so many of the babies had suffered from emotional deprivation in the early part of the study and it was impossible to determine whether the retardation was reversible and how long it would take for a child to catch up to the average It will be seen that only two of 110

Table 2 *I Q at school in relation to grading in infancy*

| I Q score at school | Grading in first year | | | | Grading in second year (different babies) | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Below 80 | 1 | 1 | 3 | 4 | — | — | 3 | 1 |
| 80–89 | 1 | 4 | 6 | 2 | — | — | — | 3 |
| 90–99 | 5 | 18 | 10 | 1 | — | 3 | 3 | — |
| 100–109 | 15 | 15 | 4 | 1 | — | 6 | 4 | — |
| 110–119 | 14 | 18 | 11 | 1 | 1 | 5 | 3 | — |
| 120 or over | 5 | 13 | 2 | 1 | — | 1 | — | 1 |
| Total | 41 | 69 | 36 | 10 | 1 | 15 | 15 | [illegible] |

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Analysis of the figures shows that there was a tendency to underestimate the potential of the babies but this could be partly explained by the fact that many had suffered emotional deprivation On the other hand only two of 110 babies thought to be average or possibly b tter had an I Q at school of below 80

Twenty one infants of a mentally defective parent had a m an I Q of 100 1 indicating the importance of not rejecting such infants as being unsuitable for adoption

It is concluded that although there never can be a high correlation between tests in infancy and the subsequent career of a child because of all the environmental factors which will in fluence him, nevertheless assessment in infancy is of considerable value for the purpose of as sessing babies for suitability for adoption

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# HEREDITARY TYROSINEMIA

## III On the Differential Diagnosis and the Lack of Effect of Early Dietary Treatment

GÖRAN BODEGÅRD JOHAN GENTZ BENGT LINDBLAD SVEN LINDSTEDT and ROLF ZETTERSTRÖM

*From the Departments of Pediatrics at Crown Princess Lovisa's Children's Hospital and Clinical Chemistry at Karolinska Hospital Karolinska Institutet Stockholm and the Department of Physiological Chemistry University of Lund Lund Sweden*

Hereditary tyrosinemia or tyrosinosis (6 12 19) is a genetically determined disorder with a clinical picture of nodular cirrhosis of the liver and multiple renal tubular disturbances Skeletal deformities muscular wasting and paresis attacks of severe pains electrolyte crises and hyperpigmentation may occur in addition to those symptoms which can be directly attributed to cirrhosis of the liver (6 12 28 32) The main biochemical characteristics are high plasma tyrosine level high urinary excretion of tyrosine and its metabolites—*p* hydroxyphenylpyruvic acid *p* hydroxyphenyllactic acid and *p* hydroxyphenylacetic acid—and a low activity of the liver enzyme which converts *p* hydroxyphenylpyruvate to homogentisate *p* hydroxyphenylpyruvate hydroxylase (EC 1 14 2 2) The disturbance of tyrosine metabolism is apparently present at birth and thus differs from the hypertyrosinemia which develops in some cases of liver cirrhosis (18) Hereditary tyrosinemia has been reported from different countries several cases from Scandinavia (6 12) and as many as 37 from a French Canadian geographic isolate (16)

The disease may start in early infancy and run an acute course ending in death from hepatic failure The course may also be more protracted and a few patients have reached adult age (6) Acute and chronic courses have been observed in the same family (6) indicating that the genotype is the same in the two types of the disease

Restriction of the intake of phenylalanine and tyrosine has been found to be beneficial to some patients with hereditary tyrosinemia (2 8 11) Since the hypothesis has been advanced that damage to the liver and kidneys may be prevented by early dietary treatment a screening procedure for the early detection of hereditary tyrosinemia has been considered to be of value (32)

We now report on the clinical and biochemical findings in one infant with hereditary tyrosinemia followed from birth and in one with transient hypertyrosinemia of early infancy The intake of phenylalanine and tyrosine was restricted as soon as the diagnosis hereditary tyrosinemia was established However the baby died at 5 ½ months of age from hepatic failure complicated by septicemia The early biochemical findings as well as the poor result of dietary treatment may have a bearing on the pathogenesis of the disease

This work has been supported by grants from Stiftelsen C ... och Thyra Svenssons Minne and the Swedish Medical Research Council (K69 19X 446-10)

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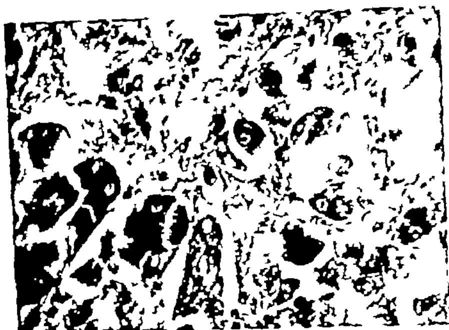


Fig. 1. Microphoto of the liver in polarized light from the patient with hereditary tyrosinemia. Double refracting crystals presumed to be tyrosine are clearly seen in the parenchymal cells. Original magnification ×480.

gether. The cortico-medullary demarcation was sharp and the renal cortex was broadened (about 8 mm) but rather pale.

Microscopical examination of the liver showed that the parenchyma was separated into irregular nodules by fibrous tissue. The number of bile ducts was increased and there was an infiltration of inflammatory cells. The liver cells showed a marked degree of fatty degeneration. There were no signs of malignancy. Crystals looking like tyrosine crystals were scattered over the liver parenchyma (Fig. 1). The tyrosine content of liver tissue was found to be 0.81 mg per g wet weight. In the kidneys a few of the glomeruli were shrunken but most of them appeared normal. There was marked dilatation of the tubules and other changes typical of tubular degeneration like atrophy and swollen cells. Dystrophic calcifications were found in the tubules and in the medullary area (Fig. 2). In the pancreas there were numerous large and hyperplastic islets (Fig. 3).

*Patient with transient hypertyrosinemia (M. Q.)*

*Case history.* A girl, the first child of healthy unrelated parents. Pregnancy and delivery were uneventful. Although she was born at term, the birth weight was only 2370 g. She received breast milk during the first week of life and was then fed a cow's-milk formula. Because of frequent loose stools she was given a high-casein formula containing 6 per cent protein from the 10th day of life. She was then continued on this formula providing a daily protein intake of about 10 g per kg. The baby gained weight and seemed to develop normally. At an age of 5 weeks she started to vomit occasionally but continued to thrive. The blood phenylalanine level was then found to be 10 mg per 100 ml. Two weeks later the plasma levels of phenylalanine and of tyrosine were 9 and 30 mg per 100 ml respectively. The baby was admitted at an age of 53 days.

*Physical examination.* The examination revealed a 7-week-old normally developed infant. The weight was 4180 g. There was no hepato-splenomegaly or neurologic abnormalities. The urine did not contain glucose or protein. The serum levels of sodium, potassium, calcium and phosphate were normal. The serum chloride concentration was 112 mmoles per l. The blood urea nitrogen concentration was 42 mg per 100 ml. The concentrations of several amino acids were elevated in plasma (Table 2). There was a very high urinary excretion of Millon reacting compounds.

*Course.* The baby was kept on the very high protein intake for one day. The feed was then changed to a cow's milk formula providing 2 g of protein per kg per day. The plasma levels of phenylalanine and tyrosine then rapidly became normal at the same time as the excretion of Millon reacting compound ceased. Within a few days the blood urea nitrogen level dropped to 10 mg per 100 ml.

After two weeks on the low protein feed the protein intake was increased to 4 g per kg per day without any reappearance of Millon reacting compounds in the urine. After discharge the baby has been seen repeatedly up to 9 months of age. She has developed normally.

## BIOCHEMICAL STUDIES

*Methods*

*Phenylalanine tolerance test.* L-Phenylalanine (E. Merck AG, Darmstadt, West Germany) in a dose of

From the findings in the two patients it is concluded that there is as yet no simple diagnostic procedure available for an early differentiation between the two conditions

## CASE REPORTS

*Patient with hereditary tyrosinemia (P IV)*

*Family history* The patient a boy was the second child of healthy unrelated parents of Finnish extraction The first child in the family is a boy who is now 6 years old who was diagnosed as a case of hereditary tyrosinemia of the chronic type at an age of 3 years Since 4 years of age he has been treated with a phenylalanine and tyrosine restricted diet which has resulted in clinical improvement The clinical and biochemical findings have been reported elsewhere (9)

*Case history* The patient was a 3500 g full term product of uncomplicated pregnancy labor and delivery Because of the family history he was transferred to the Crown Princess Lovisa's Children's Hospital immediately after birth

*Physical examination* Examination 5 hours after birth revealed an alert newborn boy of normal appearance The liver was felt 2 cm below the costal margin the surface was smooth and the consistancy was normal Otherwise physical and neurologic examination did not reveal any abnormalities

*Course* The baby was fed breast milk during the first week of life He developed mild neonatal jaundice with a peak bilirubin level of 6 4 mg per 100 ml on the 7th day of life Since hereditary tyrosinemia could not be excluded on basis of the biochemical findings (see Results) a low protein cow's milk formula was prescribed on discharge at 9 days of age The body weight intake as well as blood levels of phenylalanine and tyrosine and output of Millon reacting compounds during the course are given in Fig 4 The patient was readmitted at 4 weeks of age He had developed normally and the weight gain had been satisfactory Physical examination was normal The liver was not palpable The serum bilirubin level was 0 7 mg per 100 ml Although the result of an oral phenylalanine test was compatible with a diagnosis of hereditary tyrosinemia (Fig 5) the diagnosis was not considered so firmly established as to justify a radical restriction of the intake of phenylalanine and tyrosine The patient was discharged with the same formula and extra vitamin C

At 7 weeks of age the patient was readmitted His progress had been satisfactory Physical examination was normal except for a mild jaundice Since there was also proteinuria occasional glycosuria and generalized hyperaminoaciduria the diagnostic criteria of hereditary tyrosinemia were fulfilled At 54 days of age the baby was started on a diet based on Minafen (Trufood Ltd Wrenbury Nantwich Cheshire England) *minus* tyrosine and a low protein cow's milk formula providing him with 60 mg of phenylalanine and 60 mg of tyrosine per kg per day with a daily caloric intake of 110 kcal per kg The diet was supplemented with 100 mg of ascorbic acid per day The restricted diet caused a reduction of the serum tyrosine concentration (Fig 4) However since he did not gain weight during the following week the diet was changed to provide 130 kcal and 80 mg of tyrosine and phenylalanine respectively per kg per day The weight gain then became satisfactory and the patient was discharged after another week

At an age of 4 months the patient was readmitted The weight gain had been satisfactory and the psychomotor development was normal However despite dietary treatment there had been a marked progress of the disease The baby was more jaundiced than before There was slight abdominal distention and the liver was enlarged and had a rather firm consistancy There was no splenomegaly or signs of rickets The total serum bilirubin level was 2 7 mg per 100 ml of which 1 7 mg per 100 ml was direct reacting Bromsulphalein (5 mg per kg body weight) was eliminated at a slow rate 44 per cent was retained after 45 minutes The hemoglobin level was normal as were the white cell and differential counts The number of platelets varied between 58 000 and 132 000 per µl There was hypoglucosemia with fasting blood glucose levels varying between 25 and 35 mg per 100 ml The serum phosphate level was only 1 8 mg per 100 ml The serum concentrations of calcium sodium potassium and chloride were within normal ranges The restricted diet was continued now providing him with 75 mg per kg and day of phenylalanine and tyrosine respectively The daily protein intake was 3 6 g per kg and the caloric supply 140 kcal per kg per day

The patient was admitted for the last time at an age of 5 months The day before admittance he had developed high fever and marked abdominal distention Physical examination revealed a semicomatose moderately jaundiced infant with a peculiar odor The weight was 7150 g There was much ascites The liver and the spleen could not be palpated The total serum bilirubin concentration was 3 6 mg per 100 ml of which 3 3 mg per 100 ml were direct reacting The hemoglobin was 10 0 g per 100 ml and there was a marked leucocytosis Prothrombin was not measurable There was a marked hyperchloremic acidosis The serum concentration of phosphate was 2 4 and of calcium 7 1 mg per 100 ml The serum albumin concentration was only 1 2 g per 100 ml The level of the three main immunoglobulins were elevated (IgA was 230 IgM 225 and IgG 900 mg per 100 ml) The condition deteriorated rapidly and the patient died 30 hours after admission

*Autopsy findings* Autopsy revealed mucopurulent tracheobronchitis purulent pericarditis and peritonitis The liver was rather small and weighed 156 g The capsule was granular Numerous vividly yellow nodules up to 5 mm in diameter were irregularly distributed on the cut surface The bile ducts and the pancreas appeared normal The spleen weighed 17 5 g there was diffuse hyperemia of the pulp The kidneys were grossly enlarged and weighed 1[illegible]

Table 1 *Serum tyrosine level and urinary excretion of p-hydroxyphenylpyruvic acid p-hydroxyphenyllactic acid and p-hydroxyphenylacetic acid in the patient with hereditary tyrosinemia and in the patient with transient tyrosinemia*

The phenolic acids were determined by gas liquid chromatography

| | Age (days) | Serum level tyrosine (mg per 100 ml) | Urinary excretion in 24 hours: p-Hydroxyphenyl pyruvic acid (mg) | p-Hydroxyphenyl lactic acid (mg) | p-Hydroxyphenyl acetic acid (mg) | Total excretion (mg) |
|---|---|---|---|---|---|---|
| Patient with hereditary tyrosinemia | 61 | 0 8 | <0 3 | <0 6 | <0 05 | <1 0 |
| | 62 | 0 6 | <0 5 | 0 9 | <0 05 | <1 4 |
| | 64 | 1 3 | 1 0 | 0 6 | 0 6 | 2 2 |
| | 65 | 2 2 | 16 1 | 7 1 | 8 0 | 31 2 |
| | 173 | 8 2 | 32 0 | 96 0 | 24 0 | 152 |
| Patient with transient tyrosinemia | 54 | 29 4 | 522 | 847 | 74 | 1443 |
| | 55 | 11 6 | 218 | 4 4 | 49 | 691 |

490 nm (activating light 365 nm) The venous plasma free amino acid levels were determined according to a modification (20) of the ion-exchange chromatographic method (24) The urinary excretion of amino acids was examined by a paper chromatographic method (13) Urinary amino nitrogen was determined photometrically (13) Millon reacting compounds in the urine were estimated with tyrosine as standard ( ) p-Hydroxyphenylpyruvic acid p-hydroxyphenyllactic acid and p-hydroxyphenylacetic acid in the urine were determined by means of a gas liquid chromatographic method as earlier described (8) The acids were converted to the trimethylsilylethers of the methylesters and were separated on a column of SE 30 on Gas Chrom P 100–170 mesh Details of this procedure will be given elsewhere (9)

## RESULTS

### *Case of hereditary tyrosinemia (P W )*

*Serum tyrosine and phenylalanine levels and the excretion of Millon reacting compounds* The dietary intake of phenylalanine plus tyrosine the serum concentrations of these amino acids

Table 2 *Fasting free amino acid levels in venous plasma in the patient with hereditary tyrosinemia and in the patient with transient tyrosinemia*

The values are given in μmoles per l The normal levels are those given by Soupart (35)

| Amino acid | Case of hereditary tyrosinemia (age in days): 34 | 173 | Case of transient tyrosinemia (age in days): 54 | 57 | 96 | Normal: Mean | Range |
|---|---|---|---|---|---|---|---|
| Taurine | 69 | | 37 | 73 | 19 | 49 | 19–91 |
| Aspartic acid | 1 | | 10 | [illegible]1 | <1 | 2 | 0–9 |
| Glutamic acid | 409 | | 122 | 221 | 86 | 135 | 46–290 |
| Citrulline | 4[illegible] | | 61 | 43 | 18 | | |
| Proline | 4 6 | | 440 | 199 | 169 | 113 | 51 185 |
| Glycine | 187 | | 14 | 295 | 233 | 170 | 56–308 |
| Alanine | 397 | | 498 | 613 | 329 | 219 | 99–313 |
| Valine | 16 | 86 | 410 | 212 | 185 | 127 | 57–262 |
| Methionine | 113 | 1572 | 47 | 29 | 24 | 21 | 3–29 |
| Isoleucine | 47 | 89 | 110 | 76 | 52 | 44 | 26–94 |
| Leucine | 87 | 196 | 203 | 137 | 93 | 75 | 45–155 |
| Tyrosine | 400 | 543 | 1768 | 9 | 43 | 45 | 11 122 |
| Phenylalanine | 90 | 533 | 244 | 73 | 57 | 40 | 23–69 |
| Lysine | | | 47 | 185 | | 87 | 45–144 |



*Fig 2* Microphoto of the kidney from the patient with hereditary tyrosinemia Nephrocalcinosis in a distal tubule is demonstrated The tubular cells are swollen and degenerated Hematoxyline-eosine stain Original magnification × 120

100 mg per kg body weight was dissolved in 30–40 ml of water and given through a gastric tube Blood samples for the determination of the phenylalanine and tyrosine levels were taken by heel puncture at hourly intervals for 8 hours after the load

*Analytical methods* For determination of the tyrosine concentration in liver weighed samples were homogenized with sand and free amino acids isolated by ion exchange chromatography after percipitation of proteins with picric acid (34) Tyrosine was determined by a spectrophotofluorometric method (36) The initial protein free supernatant of serum was prepared by adding 3 ml of a 5 per cent solution of trichloroacetic acid to 50 $\mu l$ of serum The fluorescence was read in an Aminco Bowman spectrophotometer at 570 nm (activating light 460 nm) Phenylalanine was also determined spectrofluorometrically (21) on 50 $\mu l$ samples of serum The fluorescence was read at



*Fig 3* Microphoto of the pancreas from the patient with hereditary tyrosinemia There is hyperplasia of the islands of Langerhans a finding which has been reported earlier (25) Hematoxyline eosine stain Original magnification × 120

Table 1. *Serum tyrosine level and urinary excretion of p-hydroxyphenylpyruvic acid, p-hydroxyphenyllactic acid and p-hydroxyphenylacetic acid in the patient with hereditary tyrosinemia and in the patient with transient tyrosinemia*

The phenolic acids were determined by gas liquid chromatography

| | Age (days) | Serum level tyrosine (mg per 100 ml) | Urinary excretion in 24 hours: p-Hydroxyphenyl pyruvic acid (mg) | p-Hydroxyphenyl lactic acid (mg) | p-Hydroxyphenyl acetic acid (mg) | Total excretion (mg) |
|---|---|---|---|---|---|---|
| Patient with hereditary tyrosinemia | 61 | 0.8 | <0.3 | <0.6 | <0.05 | <1.0 |
| | 62 | 0.6 | <0.5 | 0.9 | <0.05 | <1.4 |
| | 64 | 1.3 | 1.0 | 0.6 | 0.6 | 2 |
| | 65 | 2.2 | 16.1 | 7.1 | 8.0 | 31.2 |
| | 173 | 8.2 | 32.0 | 96.0 | 24.0 | 152 |
| Patient with transient tyrosinemia | 54 | 29.4 | 572 | 847 | 74 | 1443 |
| | 55 | 11.6 | 218 | 424 | 49 | 691 |

490 nm (activating light 365 nm). The venous plasma free amino acid levels were determined according to a modification (20) of the ion-exchange chromatographic method (24). The urinary excretion of amino acids was examined by a paper chromatographic method (13). Urinary amino nitrogen was determined photometrically (13). Millon-reacting compounds in the urine were estimated with tyrosine as standard (72). p-Hydroxyphenylpyruvic acid, p-hydroxyphenyllactic acid and p-hydroxyphenylacetic acid in the urine were determined by means of a gas liquid chromatographic method as earlier described (8). The acids were converted to the trimethylsilylethers of the methylesters and were separated on a column of SE 30 on Gas Chrom P 100–120 mesh. Details of this procedure will be given elsewhere (9).

## RESULTS

### *Case of hereditary tyrosinemia (P. H.)*

*Serum tyrosine and phenylalanine levels and the excretion of Millon reacting compounds.* The dietary intake of phenylalanine *plus* tyrosine, the serum concentrations of these amino acids

Table 2. *Fasting free amino acid levels in venous plasma in the patient with hereditary tyrosinemia and in the patient with transient tyrosinemia*

The values are given in μmoles per l. The normal levels are those given by Soupart (35)

| Amino acid | Case of hereditary tyrosinemia (age in days): 34 | 173 | Case of transient tyrosinemia (age in days): 54 | 57 | 96 | Normal: Mean | Range |
|---|---|---|---|---|---|---|---|
| Taurine | 69 | | 37 | 73 | 19 | 49 | 19–91 |
| Aspartic acid | 1 | | 10 | 21 | <1 | 2 | 0–9 |
| Glutamic acid | 408 | | 122 | 221 | 86 | 135 | 46–290 |
| Citrulline | 42 | | 61 | 43 | 18 | | |
| Proline | 4 6 | | 440 | 199 | 169 | 135 | 31–185 |
| Glycine | 182 | | 142 | 295 | 233 | 170 | 56–308 |
| Alanine | 397 | | 498 | 633 | 329 | 219 | 99–313 |
| Valine | 167 | 96 | 410 | 212 | 183 | 127 | 57–262 |
| Methionine | 113 | 1572 | 47 | 29 | 24 | 21 | 3–29 |
| Isoleucine | 47 | 89 | 110 | 76 | 52 | 44 | 26–94 |
| Leucine | 87 | 196 | 203 | 137 | 98 | 75 | 45–155 |
| Tyrosine | 400 | 541 | 1768 | 92 | 63 | 45 | 11–122 |
| Phenylalanine | 90 | 333 | 244 | 75 | 57 | 40 | 23–69 |
| Lysine | | | 247 | 185 | | 87 | 45–144 |



*Fig 2* Microphoto of the kidney from the patient with hereditary tyrosinemia Nephrocalcinosis in a distal tubule is demonstrated The tubular cells are swollen and degenerated Hematoxyline-eosine stain Original magnification × 120

100 mg per kg body weight was dissolved in 30–40 ml of water and given through a gastric tube Blood samples for the determination of the phenylalanine and tyrosine levels were taken by heel puncture at hourly intervals for 6 hours after the load

*Analytical methods* For determination of the tyrosine concentration in liver weighed samples were homogenized with sand and free amino acid isolated by ion exchange chromatography after precipitation of proteins with picric acid (34) Tyrosine was determined by a spectrophotofluorometric method (36) The initial protein free supernatant of serum was prepared by adding 3 ml of a 5 per cent solution of trichloroacetic acid to 50 µl of serum The fluorescence was read in an Aminco-Bowman spectrophotometer at 570 nm (activating light 460 nm) Phenylalanine was also determined spectrofluorometrically (21) on 50 µl samples of serum The fluorescence was read at



*Fig 3* Microphoto of the pancreas from the patient with hereditary tyrosinemia There is hyperplasia of the islands of Langerhans a finding which has been reported earlier (25) Hematoxyline eosine stain Original magnification × 120

*Free amino acids in plasma and urine* A moderate increase in the concentration of several amino acids in plasma was found on day 34 and on day 173 (Table 2) The concentration of methionine which on the 34th day of life was slightly more elevated than that of the other amino acids reached as much as 1 572 μmoles per 1 on the day before death

A slight increase in the urinary α amino nitrogen was noted on day 34 i e 69 6 mg per day corresponding to 700 mg per g creatinine Homocystine was never detected in the urine

*Case of transient tyrosinemia (M Q)*

*Serum tyrosine and phenylalanine levels and the excretion of Millon reacting compounds* As is shown in Fig 7 the tyrosine concentration was very high in the 8th week of life (30 mg per 100 ml) when there was an excessive intake of

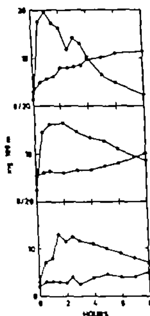


Fig 5 Phenylalanine tolerance tests in the patient with hereditary tyrosinemia The tests were performed on the 8th (upper part) 29th (middle part) and 127nd (lower part) day of life L Phenylalanine in a dose of 100 mg per kg body weight was administered through a gastric tube O——O Phenylalanine ●——● tyrosine As can be seen the tyrosine concentration rose continuously over an 8 hour period The rise became slower as the disease progressed The serum phenylalanine level returned slowly to the initial value

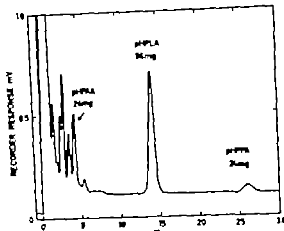



Fig 6 Gas chromatographic separation of p hydroxyphenylacetic acid (pHPAA) p hydroxyphenyllactic acid (pHPLA) and p hydroxyphenylpyruvic acid (pHPPA) excreted in the urine in the patient with hereditary tyrosinemia the day before death

tyrosine *plus* phenylalanine (about 1200 mg per kg per day) Reduction of the intake on the 55th day of life was followed by a prompt decline of the serum tyrosine concentration to within the normal range The concentration then remained normal with an intake of tyrosine *plus* phenylalanine ranging from 200 to 400 mg per kg per day Initially the serum phenylalanine concentration was high (9 mg per 100 ml) but it dropped to the normal range concomitantly with the tyrosine concentration

Immediately after admission the urinary excretion of Millon reacting compounds was very high and corresponded to about 25 per cent of the total intake of phenylalanine and tyrosine (Fig 7) The excretion was rapidly reduced to within the normal range after reduction of the intake of phenylalanine plus tyrosine to 200 mg per kg per day and remained so when the intake was increased to 400 mg per kg per day The excretion of the individual phenolic acids is shown in Fig 8 and Table 1 The proportion between the three phenolic acids was about the same as in the patient with hereditary tyrosinemia (Fig 6)

*Phenylalanine tolerance test* An essentially normal phenylalanine tolerance test was obtained on the 60th day of life when the serum

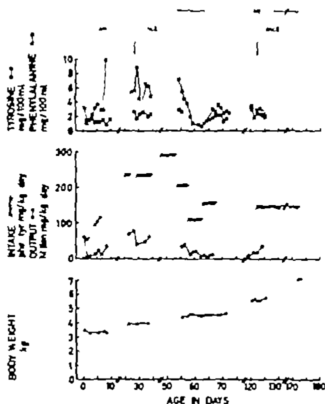



*Fig 4* Serum concentrations of tyrosine and phenylalanine dietary intake of phenylalanine *plus* tyrosine urinary excretion of Millon reacting compounds and body weight during the course in the patient with hereditary tyrosinemia Phenylalanine tyrosine restricted diet was followed by a gradual decline of the serum concentration of tyrosine

the excretion of Millon reacting compounds and the body weight are shown in Fig 4 Five hours after birth before any food had been given the tyrosine concentration was 3 2 mg per 100 ml i e slightly above the normal range for cord blood which has been reported as 0 8–1 5 mg per 100 ml (20) On the second day a normal tyrosine concentration was found but it then gradually increased to 3–4 mg per 100 ml following a successive increase in the protein intake With an intake of about 240–300 mg of phenylalanine *plus* tyrosine per kg per day the serum tyrosine concentration varied between 4 and 7 mg per 100 ml (days 25–54) The high levels on the 9th and the 30th day of life were obtained 24 hours after an oral phenylalanine tolerance test When the intake of phenylalanine *plus* tyrosine was reduced to 210 mg per kg per day and then to 120 mg per kg per day there was a gradual fall in the serum concentration of tyrosine to only 0 65 mg per 100 ml However when the daily intake was adjusted to about 160 mg of tyrosine *plus* phenylalanine per kg per day the tyrosine concentration rose again to around 3 mg per 100 ml The day before death it was 8 2 mg per 100 ml The serum phenylalanine concentration was within or slightly above the normal range, except for the last day of life when it was elevated

The urinary excretion of Millon reacting compounds followed the changes of the concentration of tyrosine in serum During the first day of life the excretion was 217 mg i e 68 mg per kg per 24 hours but on the next day only negligible amounts were excreted When the dietary intake of phenylalanine and tyrosine was gradually increased there was also a corresponding rise of the excretion of Millon reacting compounds When the intake of phenylalanine and tyrosine was reduced to a total of 120 mg per kg per day the excretion was low On no occasion except for the first day of life was more than 30 per cent of the intake of phenylalanine *plus* tyrosine recovered as Millon reacting compounds in the urine

The results of determinations of the excretion of the individual phenolic acids in urine are shown in Fig 6 and in Table 1 It is noteworthy that the total excretion of phenolic acids was less than 1 mg per 24 hours at the time when the serum tyrosine concentration had been reduced to a very low level by the restricted intake of phenylalanine and tyrosine but increased to 31 mg per day when the tyrosine concentration was only slightly above the normal range i e 2 2 mg per 100 ml

*Phenylalanine tolerance tests* The results of oral phenylalanine tolerance tests on the 8th 29th and 122nd day of life are shown in Fig 5 On all three occasions the tyrosine concentration rose continuously over an 8 hour period This rise became slower as the disease progressed The serum phenylalanine concentration returned only slowly to the initial value in this respect resembling what has been described for heterozygotes for phenylketonuria (1)

The findings in our two patients illustrate some of the difficulties of the early differential diagnosis between the two conditions. Both patients had elevated serum tyrosine levels and an increased urinary excretion of Millon reacting compounds. The relation between the three main phenolic acids was similar. Following a dietary restriction the concentration of serum tyrosine decreased rapidly in the patient with transient tyrosinemia and more gradually in the patient with hereditary tyrosinemia. A slight increase in the intake of phenylalanine and tyrosine resulted in a return to a higher blood concentration of tyrosine in this patient but not in the baby with transient hypertyrosinemia. These findings are in agreement with what has been reported to occur in other patients with hereditary tyrosinemia (8). Several phenylalanine tolerance tests were abnormal in the patient with hereditary tyrosinemia also when the serum tyrosine level was only slightly elevated. On the other hand in the baby with transient tyrosinemia the same test was normal.

Hypermethioninemia seems to be a constant finding in the acute type of hereditary tyrosinemia (10, 25, 31); in fact cases which in retrospect can be diagnosed as hereditary tyrosinemia have been described as hypermethioninemia with liver cirrhosis (27). In the present case the serum methionine concentration showed a five fold increase above normal on the 34th day of life and had increased to a very high value immediately before death. The fact that a brother of the patient has a normal methionine level (15 µmoles per l) although he is afflicted by hereditary tyrosinemia (8) may indicate that the methionine concentration in serum rises as a consequence of early severe damage to the liver.

The serum methionine concentration was only slightly elevated in the patient with transient hypertyrosinemia as part of a general elevation in the concentration of free amino acids in serum. If the main cause of transient hypertyrosinemia in a particular baby is an overload with protein it might be expected that the serum levels of most amino acids, with the exception of glycine, are elevated (33). A reduction of the protein intake will then result in a return of the concentrations to the normal range as was the case in our patient. It should be noted that on the same protein intake the serum amino acid concentrations were normal in the case with transient tyrosinemia, whereas several amino acids besides tyrosine and methionine were elevated in the patient with hereditary tyrosinemia.

Table 3. *Comparison of biochemical findings in the patient with hereditary tyrosinemia and in the patient with transient tyrosinemia before the diet was changed*

| | Patient with hereditary tyrosinemia | Patient with transient tyrosinemia |
|---|---|---|
| *Amino acids in plasma mg per 100 ml* | | |
| Tyrosine | 7.2 | 30.0 |
| Phenylalanine | 9 | 9.0 |
| Methionine | 1.7 | 0.70 |
| Others | Several elevated | Several elevated |
| Millon reacting compounds mg per kg per 24 hours | 81 | 295 |
| Aminoaciduria | | + |
| Hypoglucosemia | + | – |
| Hyperbilirubinemia | | – |
| Glucosuria | | – |

The differential diagnosis between the two types of tyrosinemia could not be made on the basis of early biochemical findings, including the pattern of phenolic acids in urine and of serum amino acids (Tables 1 and 2). The only way of obtaining an early diagnosis of hereditary tyrosinemia seems to be by a close observation for such biochemical abnormalities which indicate liver and kidney involvement, by the demonstration of abnormal phenylalanine tolerance tests and by the biochemical response to a reduction of the intake of phenylalanine and tyrosine. In our present case of hereditary tyrosinemia signs of hepatic involvement, renal tubular disturbances and abnormal carbohydrate metabolism were present already at an age of 7 weeks, whereas the baby with transient tyrosinemia seemed to be quite healthy at the

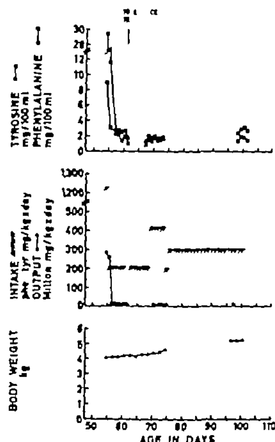



*Fig 7* Serum concentrations of tyrosine and phenylalanine dietary intake of phenylalanine *plus* tyrosine urinary excretion of Millon reacting compounds and body weight of the patient with transient tyrosinemia of early infancy There was a prompt drop of the serum tyrosine level following a reduction of the protein intake The excretion of Millon reacting compounds ceased promptly

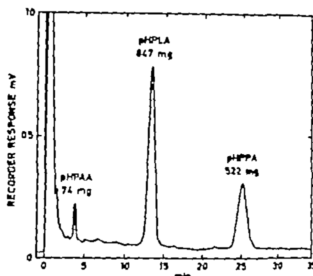



*Fig 8* Gas chromatographic separation of p hydroxyphenylacetic acid (pHPAA) p hydroxyphenyllactic acid (pHPLA) and p hydroxyphenylpyruvic acid (pHPPA) excreted in the urine on the 54th day after birth in the patient with transient tyrosinemia

tyrosine concentration had just dropped to a normal value (Fig 9)

*Free amino acids in plasma and urine* As is shown in Table 2 there was a moderate increase in the concentration of several amino acids at the time of admission After reduction of the protein intake normal values were found

The urinary excretion of α amino nitrogen was 109 mg in 24 hours (1300 mg per g creatinine) immediately after admission One day after reduction of the protein intake it was 54 mg in 24 hours and after another 10 days 30 mg (500 mg per g creatinine)

## DISCUSSION

The two patients described in this communication represent two different conditions with an increased serum concentration of tyrosine and a high urinary excretion of tyrosine and phenolic metabolites i e (*a*) the hereditary disorder tyrosinemia or tyrosinosis and (*b*) transient tyrosinemia of early infancy The second condition which is most commonly observed in infants with low birth weight (4 5 19 26) may have several causes such as delayed development of the enzyme p hydroxyphenylpyruvate hydroxylase (15) increased substrate load (3 38) and deficiency of ascorbic acid (17 19 39) There is no evidence that transient tyrosinemia is harmful (3 23 38)

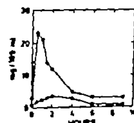



*Fig 9* Phenylalanine tolerance test in the patient with transient tyrosinemia performed on the 60th day of life i e 5 days after a reduction of the protein intake from 10 to 2 g per kg per day L Phenylalanine in a dose of 100 mg per kg body weight was administered through a gastric tube ○——○ Phenylalanine ●——● tyrosine As can be seen the response to the load was completely normal although the serum tyrosine level had been as high as 29 4 mg per 100 ml 5 days before

at 54 days of age There was a steady progress of the disease and the baby died from liver failure complicated with septicemia when he was 5½ months old The clinical course and the biochemical findings as well as the morphological changes were typical of the acute type of the disease

A 6½ year old brother suffers from the same disease of the chronic type and the two types of hereditary tyrosinemia therefore seem to belong to the same genotype

The biochemical data from the patient with hereditary tyrosinemia have been compared with those in a healthy looking baby with longstanding and pronounced transient tyrosinemia of early infancy

The patterns of amino acids in blood and of phenolic acids in urine were similar in the two patients and it is concluded that an early laboratory differential diagnosis between hereditary tyrosinemia and transient tyrosinemia may only be made by observing the biochemical response to a diet restricted in tyrosine and phenylalanine in combination with the results of phenylalanine tolerance tests

The clinical features of hereditary tyrosinemia can apparently not be attributed to a high serum tyrosine concentration or to the over production of phenolic acids the lack of effect of early restriction in the intake of phenyl alanine and tyrosine indicates a more complex pathogenesis of hereditary tyrosinemia than a primary deficiency of p hydroxyphenylpyruvate hydroxylase

## ACKNOWLEDGEMENT

Our thanks are due to Associate Professor Björn Ivemark Head of the Department of Pediatric Pathology Karolinska Institutet for histologic examination of autopsy material and for providing the microphotographs published in this report and to Dr Bo S Lindblad Department of Pediatrics Karolinska Institutet for the determination of free amino acids in plasma with ion-exchange chromatographic method

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same age although her serum tyrosine level was much higher (Table 3)

The initial similar laboratory findings in the two types of tyrosinemia make it difficult to device a simple laboratory screening test for hereditary tyrosinemia On the basis of early tyrosine determinations many cases of transient tyrosinemia would be found and require hospitalization

Determination of the plasma levels of both tyrosine and methionine might give some guide in screening for hereditary tyrosinemia at least for such cases with a tendency to run an acute course If the concentrations of both amino acids are significantly elevated in spite of low or moderate protein intake there may be reason to consider a diagnosis of hereditary tyrosinemia In our present case of hereditary tyrosinemia hypermethioninemia was present already on the 34th day of life i e before any clinical abnormalities had appeared

A diet restricted in tyrosine and phenylalanine has been reported to be beneficial in some (2 8 11 14) but not all (30) cases of hereditary tyrosinemia In our present patient such a diet was without effect Admittedly a strict regimen was not started until an age of 54 days but the serum tyrosine concentration *never exceeded 10 mg per 100 ml and the baby* had received a low protein feed since birth Since the course of hereditary tyrosinemia may either be acute or chronic (12) it has been suggested that two different types of the disorder exist Since however both types of the disease have earlier been observed in the same family (6) and since the 6 1/2 years old brother of the present case who suffers from the same disease is in a relatively good condition the two phenotypes most likely represent the same genotype No explanation can be given of the fact that the response to phenylalanine and tyrosine restriction has been found to be beneficial in the boy with the chronic type but without effect in his brother with an acute course One possibility is however that mineralization and skeletal growth improves when the acid load caused by the accumulation of phenolic acids ceases whereas the course of the liver disease remains unaffected of the diet As a consequence a restricted diet would be *effective only in those cases of hereditary tyrosinemia* with less severe liver involvement which run a more protracted course

If the primary cause of hereditary tyrosinemia is solely a deficiency of the enzyme *p* hydroxyphenylpyruvate hydroxylase it may be assumed that metabolites accumulating secondarily to the metabolic block exhibit toxic action An early restricted diet would then also be effective like in phenylketonuria The rapid progress in our present case in spite of an early institution of the restricted diet, as well as the fact that longstanding transient tyrosinemia with very high serum tyrosine level and massive excretion of phenolic acids like in the case described in this communication causes no obvious harm points to a more complex pathogenesis of hereditary tyrosinemia than a primary decrease in *p*-hydroxyphenylpyruvate hydroxylase activity (37)

Furthermore in fully developed cases of hereditary tyrosinemia there is a profund metabolic derangement which is difficult to explain as a consequence of a deficiency in *p* hydroxyphenylpyruvate hydroxylase In addition to an abnormal tyrosine metabolism there is a progressive increase in serum methionine concentration islet cell hyperplasia and renal tubular degeneration (27) There may also be slow conversion of phenylalanine to tyrosine as observed in our present patient high urinary excretion of δ aminolevulinic acid (7) abnormal carbohydrate metabolism with hypoglucosemia (14) low ceruloplasmin level (29) and hyperbilirubinemia with mainly direct reacting bilirubin

## SUMMARY

The clinical and biochemical findings in the case of an infant with hereditary tyrosinemia followed from birth have been reported The child received a low protein diet from birth and a formula diet restricted in phenylalanine and tyrosine when the diagnosis was established

*Acta Pædiat Scand* 58 49–53 1969

# STUDIES ON ERYTHRO KINETICS IN INFANCY

## XIV The relation between anaemia and haemoglobin catabolism in Rh haemolytic disease of the newborn

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By a recently developed technique using carbon monoxide analyses the haemoglobin catabolism can be measured in the newborn baby (14) In the following a study is reported in which this method was used for measuring the haemoglobin catabolism in haemolytic disease of the newborn Special interest was directed to the relationship between anaemia and increased haemolysis

## MATERIAL

Newborn infants of Rh immunized mothers were studied from birth to the beginning of the first exchange transfusion All infants had positive direct Coombs tests and their cord blood haemoglobin concentration was in all cases below 15 g 100 ml No infant was visibly oedematous They were all treated by exchange transfusion within a few hours

Cord blood was collected at birth for determination of the haemoglobin bilirubin and COHb concentrations The blood group was also determined Pulmonary excretion of CO was measured 30–60 minutes before the beginning of the first exchange transfusion At the start of this transfusion venous blood was again collected for determination of the haemoglobin bilirubin and COHb concentrations In addition the COHb concentration was determined in cord blood from 16 healthy newborn babies The mothers of these babies were non smokers and not Rh-immunized

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## METHODS

Maternal anti Rh titers were estimated with the indirect Coombs technique The concentration of haemoglobin was determined by the cyanmethaemoglobin method and that of bilirubin by the method of Michaelsson (11) COHb was estimated according to Gemzell & Wranne (7) and pulmonary excretion of CO by the technique described by Wranne (14) The prediction equation of Bratteby (3) which gives the relation between the total red cell volume per kg body weight and the venous haematocrit was used for the calculation of total body haemoglobin mass The total haemoglobin mass was calculated by means of the venous haemoglobin concentration before the first exchange transfusion The necessary conversion of Bratteby's equation was made by assuming that the mean corpuscular haemoglobin concentration was 33%

The change of the body CO pool was calculated as follows The COHb concentration of cord blood was subtracted from that of venous blood taken before the first blood exchange The difference was multiplied by the calculated total haemoglobin mass and divided by the number of hours which had elapsed before the first exchange transfusion was started One gram of haemoglobin was assumed to bind 1 34 ml CO Other CO pools besides the COHb pool were considered insignificant

## RESULTS

The relevant data of the infants with haemolytic disease are listed in the Table In the cord blood from the normal infants the COHb concentration varied between 0 3 and 0 9 % The mean

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(R Z) Crown Princess Lovisa s Children s Hospital Stockholm K Sweden

*Key words* Hereditary tyrosinemia tyrosinosis transient hypertyrosinemia dietary treatment disorders of amino acid metabolism cirrhosis of the liver

Table 1 *Clinical and laboratory data of the infants with haemolytic disease*

| | Kn | Fr | La | An 1 | Aa | Ho | Li | Se | An 2 |
|---|---|---|---|---|---|---|---|---|---|
| Birth weight g | 3990 | 2240 | 3000 | 2920 | [illegible] | 3610 | 3480 | 2960 | [illegible] |
| Duration of pregnancy days | 274 | 240 | 268 | 266 | 268 | 272 | 272 | 265 | 256 |
| Maternal indirect Coombs titer | 1:10 | 1:80 | 1:40 | 1:40 | 1:640 | 1:[illegible] | 1:20 | 1:320 | 1:80 |
| Cord blood | | | | | | | | | |
| Hb g/100 ml | 14.8 | 12.6 | 13.4 | 14.0 | 5.5 | 11.0 | 10.6 | 12.2 | 14.0 |
| Bilirubin mg/100 ml | 3.4 | 4.8 | 3.9 | 4.7 | 7.5 | 4.2 | 7.1 | 5.8 | 4.7 |
| COHb | 1.3 | 0.8 | 0.6 | 0.9 | 1.2 | 0.5 | 0.9 | 1.1 | 0.9 |
| Age at first exchange hours | 2.8 | 2.1 | 2.2 | 3.0 | 1.3 | 3.2 | 2.1 | 3.2 | 3.0 |
| Before first exchange | | | | | | | | | |
| Hb g/100 ml | 17.7 | 15.3 | 16.0 | 16.7 | 7.7 | 16.3 | 14.3 | 14.0 | 16.7 |
| Bilirubin mg/100 ml | 4.7 | 6.0 | 5.8 | 5.5 | 8.4[b] | 7.7 | 9.9 | 8.8 | 5.5 |
| COHb | 1.0 | 0.7 | 0.6 | 1.1 | 1.1 | 0.6 | 1.2 | 1.6 | 1.1 |
| Calculated total haemoglobin mass g | 54 | 26 | 36 | 37 | 11 | 44 | 36 | 30 | 37 |
| Change in body COHb pool CO μl | −182 | −28 | +0 | +119 | −26 | +54 | +149 | +194 | +119 |
| Infant's bilirubin increase mg/100 ml × hr | 0.5 | 0.6 | 0.9 | 0.4 | 1.0 | 1.1 | 1.3 | 0.9 | 1.3 |
| Pulmonary excretion of CO μl/hr[c] | 84 | 30 | 34 | 85 | 106 | 81 | 72 | 74 | 85 |
| CO formation μl/kg × hr[d] | 3.3 | 6.7 | 10.3 | 13.4 | 24 | 25 | 39 | 46 | 51 |
| Calculated haemolysis mg Hb/kg × 24 hrs | 60 | 120 | 185 | 230 | 430 | 450 | 700 | 825 | 915 |
| Haemolysis in % of normal (185 mg) | 32 | 65 | 100 | 130 | 230 | 240 | 380 | 445 | 495 |
| Haemolysis in % of calculated total Hb | 0.45 | 1.0 | 1.5 | 1.8 | 12.0 | 1.6 | 6.8 | 8.2 | 6.7 |

[a] 4.3 mg conjugated bilirubin
[b] 4.3 mg conjugated bilirubin
[c] 23°C 760 mm Hg dry (same calculation as in ref 14)
[d] STPD

by Giblett *et al* (8) however gives an idea of the maximal value for P. In their study seven adult patients with haemolytic anaemia were investigated by a number of methods. The highest average of the studied parameters in one of the patients was 9.5 times the normal corresponding approximately to 60 g Hb/24 hrs or 850 mg Hb/kg × 24 hrs. These patients had had their disease for a long time and it seems reasonable to assume that these values are representative of the maximal rate of haemoglobin production and are not exceeded in the foetus or the newborn infant.

The destruction D was estimated directly by CO analyses. THb however could only be determined indirectly from other authors' studies of the relation between total red cell volume and venous haematocrit. The prediction equation used here (3) has been shown to be valid in newborns with high haematocrit values as well as in 2- to 3-month-old infants with low haematocrit values and in normal adults. In severe anaemia such as that found in the infant Aa, the validity is less certain however. The change of THb with respect to time dTHb/dt could not be measured but will be discussed below.

At birth the daily haemoglobin metabolism has been described as follows: P = 350–400 mg/kg (6), D = 185 mg/kg (15). Thus dTHb/dt = P − D is approximately + 200 mg/kg × 24 hrs. In the normal foetus the haemoglobin concentration does not change greatly during the last weeks of pregnancy. Consequently these 200 mg are probably used for the blood volume expansion due to the growth of the foetus.

Seven of the nine infants now studied were born at 38–40 weeks gestation and had a normal birth weight. Two were born at 35 and 37 weeks; their birth weights were 2240 and 2960 g respectively. Accordingly all infants appeared to have gained weight in utero at a fairly normal rate.

Let us now assume that P and D do not change suddenly during foetal life and that dTHb/dt approximately equals the amount of haemoglobin used for growth. These assumptions are compatible with the findings in infants Kn, Fr, La and An 1. In these D ranged between 60 and 230 mg/kg × 24 hrs. The haemo-

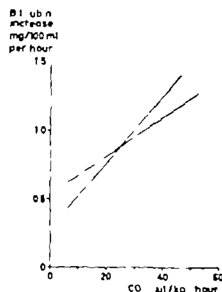



*Fig. 1.* Endogenous formation of CO compared with the rate of increase of the bilirubin concentration in plasma. The regression lines $y=0.54+0.014\ x$ and $y=0.30+0.024\ x$ are shown.

COHb concentration of the normal infants 0.5% was statistically different from that of the infants with haemolytic disease 0.9% ($p<0.001$). Among the latter the total endogenous CO formation varied between 3.3 and 51 microliters per kg body weight and per hour (μl/kg × hr).

## DISCUSSION

*Earlier studies of COHb in blood.* Bjure & Fällström in studies on healthy infants (2) and infants with haemolytic disease requiring exchange transfusion (5) found the means and S.D. of COHb in cord blood to be $1.12\pm0.14$ and $1.52\pm0.53$ respectively. In the present study lower values were found, viz. $0.5\pm0.11$ and $0.9\pm0.26$ respectively. The differences between the values in the two studies of Bjure & Fällström and the present findings may be explained partly by different methods of analysis and partly by differences in the exogenous CO and in the type of analgesia used during labour. In the present study the mothers were given a mixture of equal parts of $N_2O$ and $O_2$ during the last hours of labour. The mothers and their foetuses may then have lost excessive amounts of CO. By measuring the CO in blood as well as its pulmonary excretion the endogenous formation of CO can nevertheless be calculated (14).

*CO formation compared with other indices of haemolysis.* The endogenous CO formation was compared with the concentrations of haemoglobin and bilirubin in cord blood and with the rate at which the bilirubin concentration increased from birth to the time of the first exchange transfusion. No obvious correlation to the two first mentioned parameters was found. The rate of bilirubin increase, however, showed a correlation to the CO formation (Fig. 1).

The estimations of both variables, the bilirubin increase ($x$) and the CO formation ($y$) have large errors. Thus either of them can be taken as the dependent variable. Assuming a rectilinear relationship two regression lines were obtained: $y=0.54+0.014\ x$ and $y=0.30+0.024\ x$. The correlation coefficient was 0.77 ($p<0.02$). At present the slopes and intercepts of these two regression lines cannot be interpreted explicitly.

*Erythro-kinetics.* A complete description of the erythro-kinetics requires knowledge of the production rate (P), the destruction rate (D) and the total body mass of haemoglobin (THb). We have no direct information of P. The study

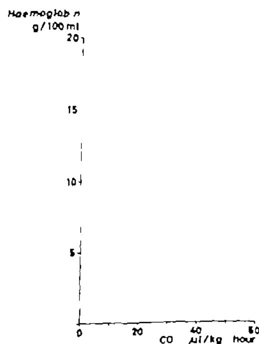



*Fig. 2.* Endogenous formation of CO compared with the concentration of haemoglobin in venous blood before the first exchange transfusion.

## SUMMARY

Endogenous formation of carbon monoxide was determined in nine infants with Rh haemolytic disease. The calculated daily haemolysis averaged 430 mg haemoglobin per kg body weight contrasting to the normal average of 185 mg/kg. No correlation could be found between the CO-formation and the haemoglobin concentration of the newborn. This confirms the clinical observation that the haemoglobin concentration is not always a reliable index of the rate of haemolysis. The findings also suggest that studies of the intrauterine haemolysis do not always give true indication of the degree of foetal anaemia.


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globin concentration in cord blood was normal or near normal compared to earlier findings in infants whose cords had been clamped early (13) Therefore dTHb/dt necessary for growth can be estimated, i e +200 mg/kg × 24 hrs Consequently P has a magnitude of 260–430 mg/kg × 24 hrs which seems reasonable

In the other five infants, D varied between 430 and 915 mg/kg × 24 hrs Since their growth also appeared normal, P should be expected to be 630–1115 mg/kg × 24 hrs Earlier it has been concluded that P is unlikely to exceed 850 mg/kg × 24 hrs Consequently the above *assumptions cannot be completely correct*

One explanation could be that dTHb/dt was smaller than +200 mg/kg × 24 hrs or even negative This would gradually decrease both the THb/kg and the haemoglobin concentration The smaller THb would tend to give a smaller D If P did not change a sort of equilibrium could then be attained If this was the case an inverse correlation could be expected between the CO formation and the haemoglobin concentration The observed data (Fig 2) showed no correlation however

Let us now consider the possibility that D or P or both changed in an irregular manner D could easily be considered to have varied The transfer of proteins of the IgG class varies and increases as the pregnancy proceeds (1) The transfer rate from mother to foetus may thus be a ratelimiting factor for red cell destruction

Such variations of the antibody transfer to the foetus and consequently of the haemolytic process seem to explain the present findings Some infants e g Se *and An 2 might have* represented an early phase in which the haemoglobin concentration was still normal or near normal (14 0 and 16 7 g/100 ml) despite a high rate of haemolysis (825 and 915 mg/kg respectively) Other infants e g An might have represented a later phase in which the haemolysis was smaller (430 mg/kg × 24 hrs) due to anaemia (7 7 g/100 ml) and a much reduced total haemoglobin mass According to reasons given above it is less probable that P would exceed 850 mg/kg A decrease of P could explain the unusually low haemoglobin concentration of infant An but does not explain the lack of correlation between the CO formation and the degree of anaemia

*Clinical aspects* The concentration of haemoglobin in cord blood is generally recognized as the most important guide for the clinical management of the newborn infant with erythroblastosis This is certainly true as far as the immediate treatment after birth is concerned For the prediction of the rate of the haemolytic process however, the haemoglobin concentration *seems less valuable according to the present findings*

*Nowadays more Rh immunized mothers lose* their foetus by stillbirth than by neonatal death The recognition and prevention of impending foetal death is thus a major problem The cause of intrauterine death and/or hydrops foetalis *in haemolytic disease is not exactly known* but foetal anaemia is by far the most probable cause It should be noted that stillbirth with the same clinical features occurs in homozygous α thalassaemia (9) In this disease severe anaemia but no hyperhaemolysis has been demonstrated

Spectrophotometric analysis of the amniotic fluid has been proved to be a very valuable aid in the clinical management of the pregnant Rh immunized mother (4) In this analysis it is probably unconjugated bilirubin that is measured i e a parameter of the haemoglobin destruction Other methods include measurements of the maternal blood COHb (12) and of the maternal endogenous CO formation (10) *These methods of course measure the* haemoglobin destruction

The present findings however indicate that anaemia and high rates of haemoglobin destruction are not necessarily parallel phenomena Since anaemia is probably the more important causative factor in intrauterine death we should try to find methods for direct measurement of the haemoglobin concentration of the foetus as a complement to the present estimations of haemoglobin destruction

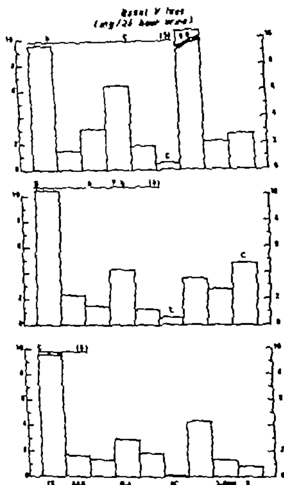


*Fig. 1.* Average basal urinary excretion of tryptophan metabolites in hepatic fibrosis and cirrhosis in childhood. A comparison is made of the basal urinary excretion of several tryptophan metabolites by a group of 6 control children, 8 children with bilharzial hepatic fibrosis and 5 children with non-bilharzial hepatic cirrhosis. Average level of excretion of each metabolite has been given for each group as mg excreted per 24-hour urine. The abbreviations representing the individual tryptophan metabolites serve to label the bars above. These abbreviations, from left to right are: *ISA*, Fraction A containing in part indoxyl sulphate; *AAG*, anthranilic acid glucuronide; *AA*, anthranilic acid; *o-AH*, ortho-aminohippuric acid; *KA*, kynurenic acid; *ACK*, Nα-acetylkynurenine; *KN*, kynurenine; *3-OHK*, 3-hydroxykynurenine; *XA*, xanthurenic acid. The letter *C* above or below a bar indicates that the height of this bar is significantly different from the corresponding one of controls ($p < 0.05$). The number of subjects in each group is given in parentheses.

load (0.5 g) with and without vitamin B supplementation (40 mg). The collection of the 24-hour urine specimens of the basal, post-tryptophan with and without vitamin B supplement was carried out as previously described (1).

The analysis of aromatic amines (fraction A, of which indican is the main constituent, anthranilic acid glucuronide, *o*-aminohippuric acid, anthranilic acid, *Nα*-acetylkynurenine and kynurenine) was performed as described by Brown & Price (4). Kynurenic acid and xanthurenic acid were determined by the fluorometric method of Satoh & Price (10). 3-hydroxykynurenine was determined in fraction E (kynurenine fraction) by the method of Brown (3). The values for these metabolites were expressed in mg/24-hour urine. Subtraction of the basal value from that present in either the post-tryptophan or the post-tryptophan supplemented with vitamin $B_6$-urine for each subject gives the response to the loading dose, i.e. yield I and II respectively, expressed as *the quantity excreted in excess of the basal level*.

Statistical analyses were made to compare the data for the studied groups of patients with data for the control subjects using the standard *t* test. *p* values of less than 0.05 were considered almost significant. It should be mentioned that although the average values of some metabolites were much higher in these groups of patients than the corresponding values in controls, yet the differences were not significant. This was due to the great individual variations in the excreted amounts of most metabolites rather than to a large error of the methods used. The analytical methods applied in this study were amongst the most sensitive ones (8).

## RESULTS

The basal, post-tryptophan values with and without vitamin $B_6$ supplementation and the metabolic responses to the tryptophan loads are graphically presented in the form of bar charts, Figs. 1, 2 and 3, respectively.

Children suffering from bilharzial hepatic fibrosis excreted more of acetylkynurenine and xanthurenic acid than controls; other metabolites are within the normal basal levels (Fig. 1). After the ingestion of the loading dose of tryptophan, these patients excreted more in mean anthranilic acid glucuronide, *o*-aminohippuric acid, acetylkynurenine and 3-hydroxykynurenine than controls, whereas the other metabolites are within the normal post-tryptophan levels (Fig. 2). However, yield I of these patients shows a significant increase in anthranilic acid glucuronide, *o*-aminohippuric

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# STUDIES ON TRYPTOPHAN METABOLISM IN BILHARZIAL HEPATIC FIBROSIS AND NON BILHARZIAL HEPATIC CIRRHOSIS IN CHILDHOOD

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Studies on tryptophan metabolism has revealed an abnormal pattern in hepatosplenic bilharziasis in adults (7) The pattern encountered was the same irrespective of the severity of the disease and whether there was gross collaterals ascites marked shrinking of the liver or not This was attributed to the inability of the liver to synthesize the enzyme proteins concerned with the degradation of tryptophan along the formylkynurenine pathway leading to the formation of niacin Since the liver parenchyma together with the liver functions are preserved in bilharzial hepatic fibrosis until the condition is far advanced (11) the diminished protein synthesis by the liver of bilharzial adult patients could be explained by the presence of a relatively advanced parenchymal dysfunction in this age group

Bilharzial patients falling within the paediatric age group might offer a better material for the study of tryptophan metabolism because they have early uncomplicated liver fibrosis Liver fibrosis is a common sequel to schistosome infection (7 11) However liver cirrhosis in children is due to multiple factors amongst which may be mentioned malnutrition viral hepatitis, obstruction to the biliary passages and congenital defects as galactosemia Wilson disease and glycogen storage disease (5) Severe and/or prolonged hepatic cellular damage is common to all known conditions predisposing to cirrhosis (9) These facts led us to carry out this comparative study between bilharzial liver fibrosis and non bilharzial liver cirrhosis in children with the aim of throwing light on the biochemical derangement in these two pathologically different categories

## MATERIAL AND METHODS

Thirteen children were studied They were classified into 2 groups according to diagnosis

1 Eight children with bilharzial hepatic fibrosis with an age ranging from 7 to 11 years 3 were males and five females The diagnosis of hepatic fibrosis was based in all cases on liver biopsy which showed fibrosis in the portal tracts Splenomegaly varying in degree was present in all cases No ascites or jaundice was present in any case The weights of all children were within the average normal for their ages and examination of the skin hair and muscles excluded subclinical protein deficiency Stool and urine analysis confirmed the bilharzial aetiology The total serum proteins done in all cases were within the normal range

2 Five children with non bilharzial hepatic cirrhosis ranging in age from 5 to 11 years 3 were males and two females A past history of jaundice was obtained in all cases and generalised oedema in two cases Signs of vitamin deficiency were present as xerosis in one case and pellagra in another case On examination jaundice was still present in two and ascites in three children The liver was very firm and enlarged from two to five fingers below the costal margin in the mid-clavicular line Liver biopsy showed post necrotic scarring with regenerating nodules The total plasma proteins ranged from 4 5 to 6 5 g/100 ml i e below the normal range

In addition 6 children of the same age group were chosen as controls

The patients have been compared with controls in respect to their ability to metabolize the tryptophan

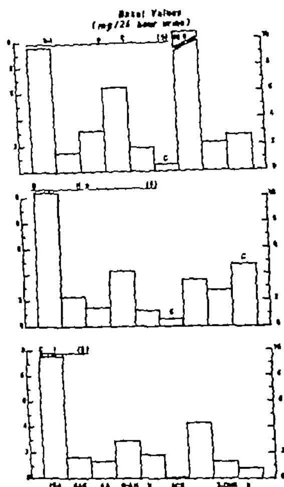



*Fig. 1.* Average basal urinary excretion of tryptophan metabolites in hepatic fibrosis and cirrhosis in childhood. A comparison is made of the basal urinary excretion of several tryptophan metabolites by a group of 6 control children, 8 children with bilharzial hepatic fibrosis and 5 children with non-bilharzial hepatic cirrhosis. Average level of excretion of each metabolite has been given for each group as mg excreted per 24-hour urine. The abbreviations representing the individual tryptophan metabolites serve to label the bars above. These abbreviations from left to right are *ISA*: Fraction A containing in part indoxyl sulphate; *AAC*: anthranilic acid glucuronide; *AA*: anthranilic acid; *o-AH*: ortho-aminohippuric acid; *KA*: kynurenic acid; *ACK*: N-acetylkynurenine; *XA*: kynurenine; *3-OHK*: 3-hydroxykynurenine; *XA*: xanthurenic acid. The letter C above or below a bar indicates that the height of this bar is significantly different from the corresponding one of controls ($p < 0.05$). The number of subjects in each group is given in parentheses.

load (0.5 g) with and without vitamin B₆ supplementation (40 mg). The collection of the 24-hour urine specimens of the basal, post-tryptophan with and without vitamin B₆ supplement was carried out as previously described (1).

The analysis of aromatic amines (fraction A, of which indican is the main constituent, anthranilic acid glucuronide, o-aminohippuric acid, anthranilic acid, N-α-acetylkynurenine and kynurenine) was performed as described by Brown & Price (4). Kynurenic acid and xanthurenic acid were determined by the fluorometric method of Satoh & Price (10). 3-hydroxykynurenine was determined in fraction E (kynurenine fraction) by the method of Brown (3). The values for these metabolites were expressed as mg/24-hour urine. Subtraction of the basal value from that present in either the post-tryptophan or the post-tryptophan supplemented with vitamin $B_6$-urine for each subject gives the response to the loading dose, i.e. yield I and II respectively, expressed as the quantity excreted in excess of the basal level.

Statistical analyses were made to compare the data for the studied groups of patients with data for the control subjects using the standard *t* test. *p* values of less than 0.05 were considered almost significant. It should be mentioned that although the average values of some metabolites were much higher in these groups of patients than the corresponding values in controls, yet the differences were not significant. This was due to the great individual variations in the excreted amounts of most metabolites rather than to a large error of the methods used. The analytical methods applied in this study were amongst the most sensitive ones (8).

## RESULTS

The basal, post-tryptophan values with and without vitamin $B_6$ supplementation, and the metabolic responses to the tryptophan loads are graphically presented in the form of bar charts Figs. 1, 2 and 3, respectively.

Children suffering from bilharzial hepatic fibrosis excreted more of acetylkynurenine and xanthurenic acid than controls, other metabolites are within the normal basal levels (Fig. 1). After the ingestion of the loading dose of tryptophan these patients excreted more indican, anthranilic acid glucuronide, o-aminohippuric acid, acetylkynurenine and 3-hydroxykynurenine than controls, whereas the other metabolites are within the normal post-tryptophan levels (Fig. 2). However, yield I of these patients shows a significant increase in anthranilic acid glucuronide, o-aminohippuric

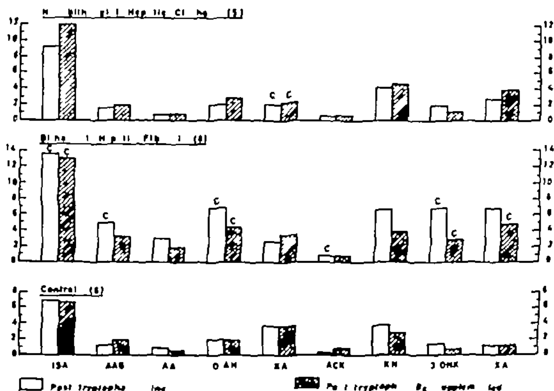



*Fig 2* A comparison is made of the urinary excretion of tryptophan metabolites by the same groups of children as shown in Fig 1 in 24 hour period following ingestion of 0 5 g L tryptophan with and without vitamin $B_6$ supplementation (40 mg) Abbreviations as in Fig 1

acid kynurenine and 3 hydroxykynurenine than the corresponding control values the other metabolites are within normal levels (Fig 3) Vitamin $B_6$ supplementation to these patients reduced the difference between the basal and the post tryptophan values Thus, the excretion pattern in yield II became quantitatively similar to that of controls (yields I and II Fig 3) Anthranilic acid being the only metabolite significantly excreted in excess to the corresponding control value (yield II Fig 3)

The basal as well as the post tryptophan values with and without vitamin $B_6$ supplementation given by the children with non bilharzial hepatic cirrhosis are within the control levels However acetylkynurenine and kynurenic acid are significantly different (Figs 1 and 2) Yield I in these children shows that anthranilic acid o aminohippuric acid and kynurenine are retained to a significant degree while the excretion of kynurenic acid is significantly less than in controls (yield I Fig 3) Vitamin $B_6$ supplementation to this group of children did not reduce the difference between the basal and the post tryptophan values (yield II Fig 3)

## DISCUSSION

*The results of this study on children with bil*harzial hepatic fibrosis differed from those reported in adults by Mousa *et al* (7) Children of the present series suffered from vitamin $B_6$ deficiency evidenced by the accumulation of anthranilic acid glucuronide *o*-aminohippuric acid kynurenine and 3 hydroxykynurenine *in the* post tryptophan urine *(yield I Fig 3)* and supported by the reduction of the difference during vitamin $B_6$ supplementation (yield II Fig 3) The accumulation of these metabolites may be due to inhibition of the $B_6$-dependent 3 hydroxykynureninase enzyme A decrease in the kynureninase would in-

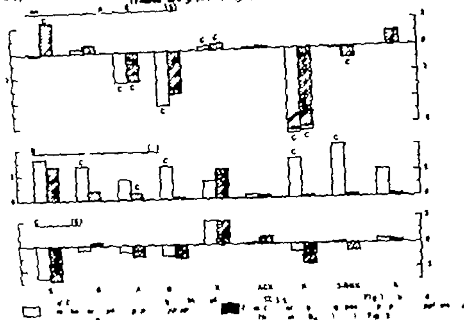


Fig. 3. Comparison of metabolic responses of children presented in Figs. 1 and 2. The values recorded represent the increase or the decrease in the excretion of various metabolites (in mg) after the ingestion of 0.5 g L tryptophan (post tryptophan values in Fig. 2 or post tryptophan pyridoxine values in Fig. 2 minus the average basal values in Fig. 1, i.e. yields I and II respectively). Abbreviations as in Fig. 1.

hibit the formation of anthranilic acid glucuronide and o-aminohippuric acid. However, the latter metabolites were significantly higher than the corresponding control values. This finding might suggest the possibility that two distinct kynureninase enzymes are involved in the cleavage of kynurenine and 3-hydroxykynurenine to anthranilic acid and 3-hydroxyanthranilic acid respectively. A similar suggestion for the transamination of kynurenine and 3-hydroxykynurenine had been given by *Korbitz et al* (6). On the other hand the abnormal pattern of tryptophan metabolites encountered in adults infected with *S. mansoni* without hepatic involvement reflected vitamin $B_6$ deficiency while that in hepatosplenic bilharziasis was characterized by retention of most metabolites (7). This latter effect was not corrected by vitamin $B_6$ supplementation. In contrast to adults, the vitamin $B_6$ deficiency encountered in children ... ...osis being most probably secondary to *S. mansoni* infection. This is because the chief metabolic product of vitamin $B_6$, 4-pyridoxic acid, was excreted in normal amounts in the basal urine of these children. Studies on pyridoxine load test of these patients is under way. Furthermore, the previous work from this laboratory has indicated that *S. mansoni* infection produced a deficiency of active pyridoxal in the infected mouse liver (2).

The elucidation of the aetiologic agent in liver cirrhosis in children is frequently difficult if not impossible (5). However, in the present series the hepatic cirrhosis was most probably due to viral hepatitis since a history of jaundice was obtained. The role of malnutrition in producing the final picture could not be excluded.

The pattern of tryptophan metabolites given by the children with non-bilharzial liver cirrhosis was found to be different from that given by those with bilharzial hepatic fibrosis. Reten

tion of most metabolites to a marked degree was the predominant feature in liver cirrhosis This is probably due to the protein malnutrition in these patients The tryptophan load given under such conditions was probably used first to establish and maintain nitrogen balance (cf 10) Evidence for protein depletion in the present series was shown by the low plasma protein levels

## SUMMARY


Study on tryptophan metabolism in eight children presenting with bilharzial hepatic fibrosis revealed a pattern reflecting vitamin $B_6$ deficiency and which could be corrected by supplementing this vitamin This finding was attributed to *S mansoni* infection rather than parenchymal involvement

Similar study carried out on five children with non bilharzial hepatic cirrhosis showed low response to a tryptophan load which was not corrected by vitamin $B_6$ supplementation

The difference between the patterns of tryptophan metabolites in bilharzial fibrosis and non bilharzial cirrhosis in children might be due to bilharziasis in the former group and to deranged protein synthesis in the latter


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# BILE ACID EXCRETION AND MALABSORPTION IN INTRAHEPATIC CHOLESTASIS OF INFANCY ( NEONATAL HEPATITIS )

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The main clinical features of the syndrome of intrahepatic cholestasis of infancy are obstructive jaundice with acholic stools hepatomegaly and a poor nutritional condition Occasionally there is also pruritus hemorrhagic disease and retardation of growth (for review cf 9) Symptoms of the disease are usually recognized in the second to eight week of life and its course is variable Approximately half of the infants affected by the disorder will make an apparently complete recovery (23) Roughly 25 per cent will die in the first few months of life and in another 25 per cent the condition has a more protracted course with cirrhosis of the liver (23) Microscopical examination of the liver shows intracellular and intracanalicular bile stasis and the presence of numerous multinucleated giant cells apparently formed from the parenchymal cells (56) In later stages there may also be fibrosis of the liver (15 26 51) The syndrome differs both from extrahepatic atresia of bile ducts from which it is frequently indistinguishable (37) and from bacterial and viral hepatitis of infancy (47) It also differs from other conditions which may be associated with prolonged obstructive jaundice such as erythroblastosis due to blood group incompatibility (50 57 67) cystic fibrosis (22, 62) galactosemia (4) and hereditary tyrosinemia (6)

The etiology of the syndrome of intrahepatic cholestasis of infancy is uncertain There is little evidence that the agent of homologous serum hepatitis or the virus of infectious hepatitis is the cause of the disorder (7) A familial incidence has been reported repeatedly (12 14 26 28 34) and inheritance due to an autosomal recessive gene has been proposed as the cause of the disease (31) At the present time it cannot be concluded whether the syndrome constitutes a clinical entity or if it includes several etiologically different disorders The uncertainty about etiology and pathogenesis is illustrated by the various alternative names which have been given to the condition (14 26 34) such as neonatal hepatitis (23 55) and giant cell hepatitis (12) Cases associated with atrophy of the interlobular bile ducts have been called atresia of the intrahepatic bile ducts (2 27)

Studies of the excretion of cholic acid 24-$^{14}$C in four babies with intrahepatic cholestasis of infancy will be reported in this communication In three of the cases the diagnosis was established further by operative cholangiography and histological examination of the liver The excretion of bile acids has been compared with the results of liver function tests and studies of intestinal absorption

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tion of most metabolites to a marked degree was the predominant feature in liver cirrhosis This is probably due to the protein malnutrition in these patients The tryptophan load given under such conditions was probably used first to establish and maintain nitrogen balance (cf 10) Evidence for protein depletion in the present series was shown by the low plasma protein levels

## SUMMARY

Study on tryptophan metabolism in eight children presenting with bilharzial hepatic fibrosis revealed a pattern reflecting vitamin $B_6$ deficiency and which could be corrected by supplementing this vitamin This finding was attributed to *S mansoni* infection rather than parenchymal involvement

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The difference between the patterns of tryptophan metabolites in bilharzial fibrosis and non bilharzial cirrhosis in children might be due to bilharziasis in the former group and to deranged protein synthesis in the latter

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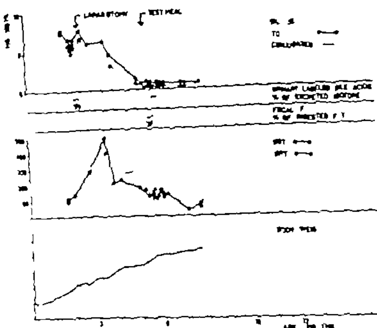


*Fig. 3.* Patient I. J. For explanation see Fig. 2.

mainly polyunsaturated fatty acids were given. The protein intake was 2.8 g per kg per day.

Until 8 months of age there was no relapse of jaundice. Psychomotor development was normal as was the rate of growth (Fig. 1). Weight gain was rather slow (Fig. 3).

There were no hematological abnormalities. At 8 months of age the plasma methionine concentration was slightly elevated. The blood ammonia concentration was normal.

## Case 3

I. B. A boy, the only child of healthy unrelated parents. Pregnancy, labor and delivery were without complications. The baby was born 2 weeks before term; birth weight was 2940 g, birth length 48 cm. There was a moderate neonatal jaundice persisting for 10 days, the peak serum bilirubin concentration was 1. 9 mg per 100 ml. The mother was Rh negative and the infant Rh positive but there was no evidence of isoimmunization. In the first week of life a cardiac systolic murmur was found. After cardiac work up at 4 months of age a diagnosis of aortic stenosis and mild mitral insufficiency was made. There were never any signs of cardiac failure.

At months of age the patient developed jaundice in conjunction with acute gastroenteritis associated with a pale rash. Since the jaundice persisted he was admitted one month later. There was a moderate enlargement of the liver. The hyperbilirubinemia was due almost quantitatively to direct reacting bilirubin. Laparotomy with cholangiography showed normal extrahepatic bile ducts. The gall bladder contained bile of normal appearance. Histological examination of the liver showed pronounced intracanalicular bile stasis. No real giant cells were found but some of the parenchymal cells contained two or more nuclei. There was no fibrosis.

After laparotomy the jaundice gradually disappeared but steatorrhea remained. At 6 months of age jaundice recurred, the total serum bilirubin concentration increasing to a higher level than previously. The baby then remained markedly jaundiced during 2 months of observation. At 6 months of age the fat intake was reduced to 2.6 g per kg per day consisting of polyunsaturated fatty acids. The protein intake was 2.7 g per kg per day and the daily caloric supply 150 kcal per kg. There was a slight retardation of growth (Fig. 1). Psychomotor development was normal.

There was a mild normochromic anemia. Target cells were found in repeated blood smears. During the first episode of obstructive jaundice there was a moderate hypermethioninemia.

Bilirubin concentrations and body weight during the course of the disease are shown in Fig. 4.

## Case 4

M. W. A boy, the only child of healthy unrelated parents. He was born in the 43rd week after an uneventful pregnancy. Delivery was normal; birth weight 3100 g and birth length 52 cm. The blood group of the mother was 0 and of the baby B, both were Rh negative. There was no evidence of isoimmunization.

In the first week of life the baby started to vomit and during the second week jaundice developed. One week later he developed hepatomegaly and got symptoms of hemorrhagic disease. Prothrombin time was

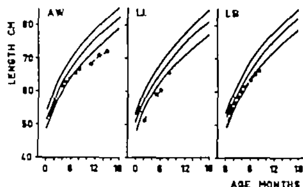



*Fig 1* Growth chart for three of the patients with intrahepatic cholestasis (*dotted lines*) The lines indicate the mean and the 10th and 90th percentiles (33)

## CASE REPORTS

### *Case 1*

A W A boy the first child in the family The mother was jaundiced and had pruritus during the last month of pregnancy Otherwise the family history was non contributory The patient was the 3750 g product of uncomplicated labor and delivery with birth length 51 cm The mother was of blood group 0 and the infant of group A but there was no evidence of iso immunization

At 6 weeks of age the baby developed obstructive jaundice which persisted unchanged until 3 months of age when the bilirubin concentration decreased However at 6 months of age jaundice recurred He then also had itching and the stools became loose and clay colored There was a moderate hepatomegaly Since cholestasis persisted laparotomy was performed to exclude occlusion of extrahepatic bile ducts Cholangiography disclosed normal extrahepatic bile ducts The gall bladder contained bile of normal appearance Histological examination of the liver showed intracellular and intracanalicular bile stasis the presence of numerous giant cells and some infiltration of lymphocytes

After laparotomy there was failure to thrive persistant jaundice marked steatorrhea hyperlipemia and hypermethioninemia (highest plasma methionine concentration 238 $\mu$mol per 1) A diet high in carbohydrates but low in fat and proteins was instituted at 8 months of age However since the general condition was unchanged and since there was a marked retardation of growth (Fig 1) the protein intake was increased from 1 7 to 2 7 g per kg per day at 13 months of age Vitamin A and D were administered by the parenteral route During subsequent months jaundice diminished and weight gain improved The plasma methionine concentration decreased but hyperlipemia persisted Psychomotor development was normal

There was a mild normochromic anemia and a slight macrocytosis Some target cells were constantly seen in the blood smear from 6 months of age The white cell and differential counts were normal Prothrombin time was normal The blood ammonia concentration was not elevated

The body weight during the course of the disease is shown in Fig 2 From about an age of 12 months there has been an increasing amount of hair over the sides of the face trunk and limbs

### *Case 2*

I J A girl the only child of a 16 year old mother Family history was non contributory There was toxemia of pregnancy The baby was born 4 weeks before term Delivery was normal and the birth weight was 2120 g birth length 44 5 cm There was no blood group incompatibility Neonatally the baby had attacks of cyanosis and a congenital heart disease was diagnosed At 2 months of age angiocardiography and heart catheterization disclosed ventricular septal defect and supravalvular pulmonary stenosis There were never any signs of cardiac failure

During the course of a mild upper respiratory tract infection at 6 weeks of age the baby developed obstructive jaundice The urine was dark and the stools acholic At operative cholangiography 1 month later the extrahepatic bile ducts were normal The gall bladder contained bile of normal appearance Histological examination of the liver showed fibrosis around the portal tract numerous giant cells and intracellular and intracanalicular bile stasis

A moderate hepatomegaly was found at 3 months of age Since there was an increase of serum transaminases even though the baby became less jaundiced treatment with prednisolon was instituted at 4 months of age and maintained for 7 weeks There were signs of hemorrhagic disorder with repeated episodes of mild melena occasionally also of bleeding from the nose Prothrombin time remained however normal At 6 months of age jaundice disappeared completely However since the patient still had steatorrhea the fat intake was reduced to 3 2 g per kg per day and

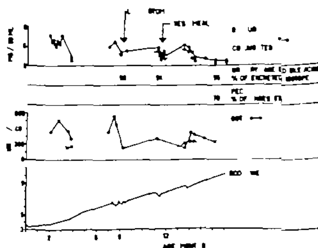


*Fig 2* Patient A W Body weight and changes of serum concentration of bilirubin transaminases (GOT GPT) (38) urinary bile acid excretion and fecal fat excretion during the course of the disease

The serum activities of alkaline phosphatases leucine aminopeptidase (LAP) and γ-glutamyl transpeptidase (GT) are given in Table 2. In case A W alkaline phosphatases activity was normal for a long period of clinical cholestasis. Only in case I J were both LAP and GT activities elevated during the whole course of the disease. In case L B there was a gradual increase in the serum activities of both enzymes when jaundice recurred.

*Various other laboratory data*

As can be seen from Table 2 there was a marked elevation of the fasting serum triglyceride concentration and a slight hypercholesterolemia in case A W. The serum cholesterol level was also slightly elevated in case I J. The total protein and albumin levels in serum were normal in all 4 patients. Prothrombin time was initially prolonged in cases I J and M W and parenteral administration of vitamin $K_1$ produced a return to normal values in both patients.

The following investigations gave normal results in the 4 patients: serum concentrations of calcium and inorganic phosphate, sweat chloride concentration by means of pilocarpin iontophoresis, chymotrypsin activity in feces, tyrosine concentration in blood, Millon reacting compounds in urine and vitamin $B_1$ and folic acid concentrations in serum.

In all 4 patients the results were negative for the following tests: hemagglutination inhibition test for rubella, Sabin–Feldman dye test, complement fixation test for toxoplasmosis, listeria and cytomegalo-virus, attempted isolation of cytomegalo-virus, microscopical examination for intracellular inclusion bodies in cells in the urine and serological tests for syphilis.

## STUDIES OF INTESTINAL ABSORPTION

### Methods

*Testmeal*

At ages given in Figs. 5 a testmeal consisting of 0.5 g xylose and 8 ml cream (fat content 12 per cent) per kg body weight respectively and of 20 g glucose was given by stomach tube in the early morning 5 hours after the last meal (45). Immediately before the testmeal 7500 I.U. of vitamin A palmitate (Ido-A® Ferrosan) per kg body weight was administered through the stomach tube. Capillary blood was drawn at regular intervals for 5 hours after the testmeal for the determinations of glucose (29), xylose (40), triglycerides (41) and vitamin A (35).

*Fat balance*

The patients were kept on a fixed fat intake calculated to account for 30–45 per cent of the caloric supply. After 2–3 days daily collection of feces for a 6-day period was started except for a few occasions when it was not possible to continue for more than 4 days. The percentage fat not being absorbed was calculated after the determination of the fat excreted in feces (36).

Table 1. *Liver function tests*

| Patient | Galactose tolerance test (63) (t ½ min) | Bromsulphalein tolerance test (24) (% retention after 45 min) |
|---|---|---|
| A W | 15 (38 wks) | 19 (34 wks) |
| I J | 9 (22 wks) | 12 (24 wks) |
| *L B* | *8 (20 wks)* | *27 (24 wks)* |
| M W | 12 (7 wks) | 8 (7 wks) |
| Normal range | <17 | <5 |

Ages when the test was performed in parentheses.

### Results

*Testmeal*

The blood concentrations of glucose, xylose, triglycerides and vitamin A during the testmeal are given in Fig. 6. In all 4 patients there was a normal rise of the blood glucose level but there was a delayed return to the original level. Xylose absorption seemed to be normal in patients A W and L B but slightly impaired in I J and M W. After the testmeal the concentration of triglycerides remained unchanged in the patients A W and I J while in patients L B and M W there was a moderate increase. Before the testmeal the serum concentration of vitamin A was very low in patients A W and L B. After oral administration of vitamin A there was no significant increase in the serum

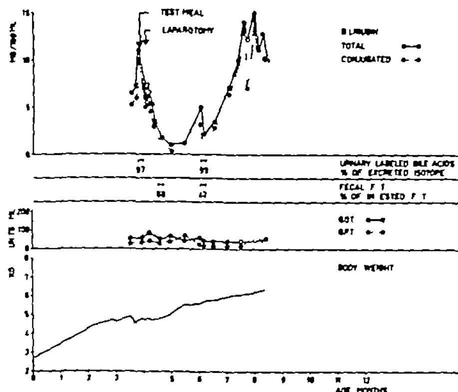



*Fig 4* Patient L B For explanation see Fig 2

markedly prolonged but became normal after parenteral administration of vitamin K Since jaundice progressed the stools were noted to lack pigment and the urine was dark The patient was admitted for operative cholangiography at an age of 4 weeks and at the same time a carbohydrate rich diet was instituted However during the week after admission there was a rapid decline of the total serum bilirubin concentration and urinary excretion of bile pigments ceased at the same time The course thus excluded a diagnosis of bile duct atresia and laparotomy was not performed There was no hyperlipemia and the plasma concentration of methionine was normal

At 2 months of age the patient was in excellent condition and had a satisfactory weight gain (Fig 5) He was on a high caloric diet restricted in saturated fatty acids providing him with 3 0 g of protein per kg per day

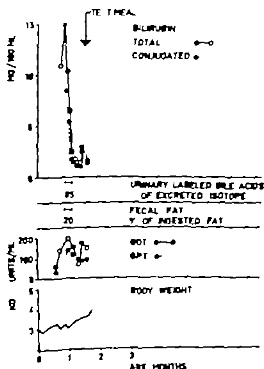



*Fig 5* Patient M W For explanation see Fig 2

## LABORATORY DATA IN THE PATIENTS STUDIED

### *Tests of liver function*

The results of bromsulphalein retention tests are given in Table 1 Abnormal values were obtained in all patients Galactose tolerance tests were normal in all four patients (Table 1)

The serum concentration of total and direct reacting bilirubin and the serum activities of glutamic oxalacetic and glutamic pyruvate transaminases (GOT and GPT) (38) during the course of the disease in the four patients are shown in Figs 2–5 Serum transaminase activities were elevated when the patients were jaundiced in cases A W I J and M W whereas normal values were found in case L B

| Normal range | |
|---|---|
| Concentration | Age group (wks) |
| 00–700 | 4 52 |
| 25 150 | |
| <15 | 1 52 |
| 85 255 | 1 52 |
| 200–300 | 0–20 |
| 15 50 | 24–52 |

For separation of the unhydrolyzed urinary bile acids urine was lyophilized and extracted with ethanol at room temperature for three hours After filtration and evaporation aliquots were used for TLC chromatography The radioactive spots were located by autoradiography

After alkaline hydrolysis of lyophilized urine the unconjugated bile acids were separated by reversed phase partition chromatography on Hyflo Super-Cel columns and by TLC (44) Using these methods and with the amount of labeled cholic acid administered more than 5 conversion into one labeled metabolite was necessary to allow the detection of this metabolite

*Fecal isotope fractionation*

Feces were homogenized in 80 per cent ethanol with an Ultra Turrax homogenizer (Janke and Kunkel K G Staufen Germany) The homogenate and the diaper contaminated with feces were combined and refluxed twice for three hours with 80 per cent ethanol The residue was extracted for 48 hours with boiling chloroform/methanol 1 1 (19) Total fecal isotope excretion was calculated as the sum of isotope in the combined ethanol extracts and the chloroform—methanol extracts

*Isotope determination*

Aliquots of the extracts were subjected to oxygen flask combustion and the $^{14}C$ in the resultant carbon dioxide was determined by liquid scintillation (16)

*Results*

*Analysis of gall bladder bile*

The concentrations of total solids bile acids cholesterol phospholipids and bilirubin in gall bladder bile obtained from patients A W I J and L B are given in Table 3 For comparison values for these substances in hepatic bile from adults and from gall bladder bile of infants are given in the same table In all three patients the concentration of total solids was

Table 3 *Composition of gallbladder bile obtained at laparotomy*

| | Bile concentration of | | | | |
|---|---|---|---|---|---|
| | Total solids (mg/100 ml) | Bilirubin (mg/100 ml) | Cholesterol (mg/100 ml) | Phospholipids (mg/100 ml) | Bile acids (mmol/l) |
| *Patient* | | | | | |
| A W | 990 | 6 9 | <1 | 2 0 | <0 01 |
| I J | 1470 | 16 0 | <1 | 4 5 | 0 17 |
| L B | 1150 | 12 7 | <1 | 6 5 | 0 07 |
| *Normal values* | | | | | |
| Hepatic bile obtained at operation of adults for cholelithiasis (43) | | | | | |
| Mean | 4303 | [illegible] | 311 | 8.3 | |
| s e | 113 8 | 4.2 | 14 1 | 43 4 | |
| Hepatic bile collected during intact enterohepatic circulation from adults (64) | | | | | |
| Range | | | | | 3–45 |
| Bile obtained from duodenum of infants by intubation (age group 10 days–7 months) (49) | | | | | |
| Range | | | | | 7 6–19 0 |

not detected by TLC chromatography



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Table 2 *Various laboratory data*

| | Patient | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A W | | | | I J | | | | | L B | | | | | M W | | |
| Age wks | 30 | 44 | 58 | 68 | 16 | 20 | 24 | 28 | 35 | 16 | 22 | 24 | 30 | 37 | 4 | 6 | * |
| Cholesterol mg/100 ml | 253 | | 219 | | | 208 | 174 | | 230 | | 136 | 106 | 210 | | 177 | 171 | |
| Triglycerides mg/100 ml (41) | | 300 | 300 | | | 94 | | | 126 | | | 115 | 115 | | 144 | 53 | |
| Alkaline phosphatases units (10) | 14 1 | 10 4 | | 26 | 24 0 | | | 27 | | 16 6 | | | 26 | | | | |
| Leucine aminopeptidase units (52) | | | 150 | 170 | | 560 | 625 | 240 | 750 | | 65 | 215 | 310 | 285 | 235 | 245 | 1 |
| γ Glutamyl transpeptidase units (61) | | | 60 | 50 | | 2600 | 1920 | 1220 | 1244 | | | 58 | 98 | 82 | 573 | 398 | 1 |

concentration in cases A W I J and L B In case M W there was a normal concentration of vitamin A in the blood but this test was performed in the period of recovery

*Fat balance studies*

The percentage of ingested fat recovered in feces in the 4 patients is given in Figs 2–5 Fat absorption was markedly impaired in cases A W I J and L B In the patient I J 30 per cent of ingested fat was excreted even when the patient was anicteric The lack of correlation between steatorrhea and the severity of jaundice is also demonstrated from Fig 2 (case A W) In case M W steatorrhea was rather mild

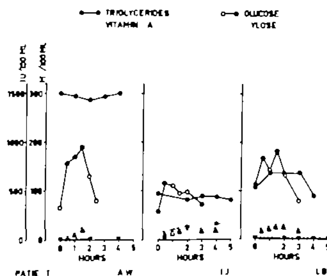



*Fig 6* Blood concentration of triglycerides vitamin A glucose and xylose after testmeal

## COMPOSITION OF BILE AND EXCRETION OF BILE ACIDS

### *Methods*

Bile was obtained from the gall bladder at laparotomy The bile concentrations of total solids bilirubin cholesterol and phospholipids were determined (43) The conjugated bile acids in bile were separated by thin layer chromatography (TLC) using a phase system consisting of *n* butanol water acetic acid 50 5 5 (21) and revealed by spraying with phosphomolybdic acid The amounts in the spots were estimated by comparing their intensity with those obtained by chromatography of known amounts of reference substances After hydrolysis of bile the unconjugated bile acids were separated by TLC according to Eneroth (18)

1 μc sodium cholate 24 C (specific activity 47 5 μc per mg) (New England Nuclear Corp Boston Mass) was dissolved in 1 0 ml saline and autoclaved before intramuscular injection 24 hour urine collection was done with adhesive disposable urine collector bags for at least four days If urine had escaped from the bag the paper diaper was saved for extraction The diapers containing feces were collected daily for at least four days All samples were stored at −27 C

*Urine isotope fractionation*

The urinary bile acids were extracted and fractionated (3) Glycine conjugates and unconjugated bile acids were extracted with ethyl acetate and separated by column chromatography Conjugates of cholic acid other than with glycine that remained in the urine after extraction with ethyl acetate were extracted with butanol No isotope was detected in urine after butanol extraction

Urinary bile acids in the diapers were extracted by refluxing in 80 per cent ethanol The total isotope was calculated as the sum of isotop in the ethyl acetate and butanol extracts of urine and the 80 per cent ethanol extracts of the diapers

| Normal range | |
|---|---|
| Concentration | Age group (wks) |
| 100–200 | 4–52 |
| 25–150 | |
| 15 | 1 52 |
| 85–255 | 1 52 |
| 200–300 | 0–20 |
| 15–50 | 24–52 |

For separation of the unhydrolyzed urinary bile acids urine was lyophilized and extracted with ethanol at room temperature for three hours After filtration and evaporation aliquots were used for TLC chromatography The radioactive spots were located by autoradiography

After alkaline hydrolysis of lyophilized urine the unconjugated bile acids were separated by reversed phase partition chromatography on Hyflo Super Cel columns and by TLC (44) Using these methods and with the amount of labeled cholic acid administered more than 5% conversion into one labeled metabolite was necessary to allow the detection of this metabolite

### Fecal isotope fractionation

Feces were homogenized in 80 per cent ethanol with an Ultra Turrax homogenizer (Janke and Kunkel K G Staufen Germany) The homogenate and the diaper contaminated with feces were combined and refluxed twice for three hours with 80 per cent ethanol The residue was extracted for 48 hours with boiling chloroform/methanol 1 1 (19) Total fecal isotope extraction was calculated as the sum of isotope in the combined ethanol extracts and the chloroform—methanol extracts

### Isotope determination

Aliquots of the extracts were subjected to oxygen flask combustion and the C in the resultant carbon dioxide was determined by liquid scintillation (16)

## Results

### Analysis of gall bladder bile

The concentrations of total solids bile acids cholesterol phospholipids and bilirubin in gall bladder bile obtained from patients A W I J and L B are given in Table 3 For comparison values for these substances in hepatic bile from adults and from gall bladder bile of infants are given in the same table In all three patients the concentration of total solids was

Table 3 *Composition of gallbladder bile obtained at laparotomy*

| | Bile concentration of | | | | |
|---|---|---|---|---|---|
| | Total solids (mg/100 ml) | Bilirubin (mg/100 ml) | Cholesterol (mg/100 ml) | Phospholipids (mg/100 ml) | Bile acids (mmol/l) |
| *Patient* | | | | | |
| A W | 980 | 6 9 | < 1 | 2 0 | < 0 01 |
| I J | 1470 | 16 0 | < 1 | 4 5 | 0 17 |
| L B | 1150 | 12 7 | < 1 | 6 5 | 0 07 |
| *Normal values* | | | | | |
| Hepatic bile obtained at operation of adults for cholelithiasis (43) | | | | | |
| Mean | 4503 | 88 | 311 | 823 | |
| s E | 113 8 | 4 2 | 14 1 | 43 4 | |
| Hepatic bile collected during intact enterohepatic circulation from adults (64) | | | | | |
| Range | | | | | 3–45 |
| Bile obtained from duodenum of infants by intubation (age group 10 days–7 months) (49) | | | | | |
| Range | | | | | 7 6–19 0 |

not detected by TLC chromatography

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Table 2 *Various laboratory data*

| | Patient | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A W | | | | I J | | | | | L B | | | | | M W | |
| Age wks | 30 | 44 | 58 | 68 | 16 | 20 | 24 | 28 | 35 | 16 | 22 | 24 | 30 | 37 | 4 | 6 |
| Cholesterol mg/100 ml | 253 | | 219 | | | 208 | 174 | | 230 | | 136 | 106 | 210 | | 177 | 171 |
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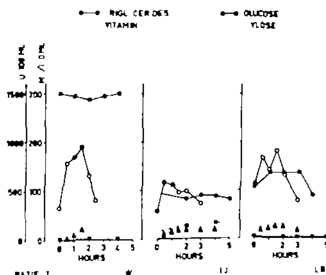



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rithmically against time straight lines were obtained The rate of decrease of isotope/mg creatinine in patients during the icteric phase with practically no fecal isotope excretion were in accordance with values predicted from the determined half time of cholic acid

*Chemical nature of labeled urinary bile acids*

Aliquots of urine excreted during the four days after administration of cholic acid 24-$^{14}$C were pooled lyophilized and the labeled bile acids extracted The labeled bile acids were chromatographically separated before and after hydrolysis Column chromatography of the hydrolyzed labeled bile acids excreted by all four patients at the ages indicated in Fig 7 only gave labeled bile acids at one position i e at the place of cholic acid TLC chromatography with different phase systems revealed only one labeled compound with the mobility of cholic acid

The ethanol extracts of lyophilized urine were analyzed with TLC for types of conjugates of cholic acid excreted A representative chromatogram is shown in Fig 8 Labeled compounds were found at the place of glycocholic and taurocholic acid and at three other positions i e one band moving faster and two bands slower than taurocholic acid The same five conjugate bands were seen in all patients however the quantitative distribution of isotope in these bands varied greatly

Aliquots of each 24 hour urine specimen were extracted with ethyl acetate and then with butanol in order to determine the excretion of unconjugated cholic acid and the per cent of conjugates present as glycocholic acid No isotope was detected in urine after butanol extraction When the ethyl acetate extracts were chromatographically separated glycocholic acid and cholic acid were the only labeled bile acids to be found The 4 other conjugates besides glycocholic acid were not extracted with ethyl acetate but with butanol The results are summarized in Fig 9 Unconjugated bile acids were only detected in one 24 hour specimen from

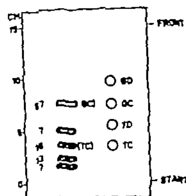



Fig 8 Thin layer chromatographic separation of labeled urinary bile acids excreted during 4 days after administration of cholic acid 24-$^{14}$C to patient I J at an age of 10 weeks Phase system n butanol water acetic acid 50 5 5 Ethanolextracts of lyophilized urine Reference substances taurocholic acid (*TC*) taurodeoxycholic acid (*TD*) glycocholic acid (*GC*) and glycodeoxycholic acid (*GD*) Open circles represent spots detected by spraying with phosphomolybdic acid The cross-hatching represents bands detected by autoradiography The percentage distribution of isotope between the five bands is indicated with figures The major bands corresponding to the glycine and taurine conjugates of cholic acid are indicated by the letters in parentheses

patient I J at 10 weeks of age In patients I J L B and M W 36–75 per cent of the excreted cholic acid was in the form of glycocholic acid In patient A W glycocholic acid accounted for only 21–26 per cent of the total conjugates

## DISCUSSION

*Diagnosis*

The diagnostic criteria of the disease recognized in the four patients described in this communication fulfill those of the syndrome of intrahepatic cholestasis of infancy (14 26 34) neonatal hepatitis (23 55) or giant-cell hepatitis (12) Giant cells were demonstrated histologically in two of the patients In the third patient there were no real giant cells in the liver but several cells contained two or more nuclei In the case of the fourth patient, no liver biopsy was performed

The presence of a congenital heart defect in two of the patients with cholestasis (I J and

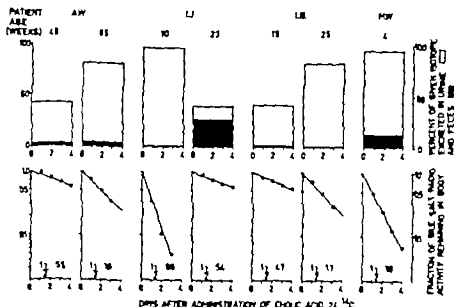


Fig 7 Upper part Per cent of isotope excreted in urine and feces during four days following administration of cholic acid 24 $^{14}$C Lower part Semilogarithmic plot of radioactivity remaining in the body versus time and the graphically obtained values for half time (t ½) for the rate of disappearance of injected cholic acid 24 C

very low The concentration of bilirubin was approximately twice as high as in the serum Phospholipids were present in trace amounts and cholesterol in so low a concentration that it not could be measured with the method used

As can be seen from Table 3 the total concentration of bile acids was extremely low No bile acids were detected in patient A W Glycine and taurine conjugates of cholic and chenodeoxycholic acids were demonstrated in patients I J and L B Neither deoxycholic nor lithocholic acid was detected after TLC separation of hydrolyzed bile

*Excretion of isotope after administration of cholic acid 24 $^{14}$C*

The percentage of excreted isotope appearing in the urine during the 4 days following administration is shown in Figs 2–5 and 7 upper part When the patients were jaundiced practically all of the labeled bile acids excreted appeared in the urine During the anicteric period in patient I J the urinary excretion decreased to 31 per cent In patient M W the study was performed during the period of rapid decrease of bilirubin concentration in the serum The relatively high fraction of isotope excreted in feces was due to the fecal isotope excretion during the third and fourth day after administration of labeled cholic acid

As shown in the upper part of Fig 7 the recovery of isotope varied during the four days following administration of cholic acid 24 $^{14}$C The extracts of the diapers not contaminated with feces contained 5–35 per cent of the urinary labeled bile acids As the collection of urine into the urine collector bags was incomplete the low fecal isotope excretion obtained in some patients may not be significant but due instead to contamination of feces with urine

Semilogarithmic plots of fractions of bile salt radioactivity remaining in the body versus time are shown in the lower part of Fig 7 The plots revealed a straight line relationship The half time of cholic acid was determined graphically and the values given in Fig 7 The rate of elimination of cholic acid varied between patients and in the same patient during different periods of the disease (half time of cholic acid between 0 6 and 5 5 days) In patient A W and L B the excretion rate was determined twice during the icteric phase In both cases the elimination rates were slower at the beginning of the disease The different recoveries of isotope obtained during the four days following administration of cholic acid 24 $^{14}$C may be explained by the differences in elimination rates of cholic acid

In all patients 24 hour excretions of creatinine were determined When the cpm of bile acid per mg creatinine was plotted semiloga

dehydroxylation and deoxycholic acid is rarely detected in children under the age of one year (17 49). During the first days after birth the bile acids are mainly conjugated with taurine but the proportion of glycine conjugates rapidly increases and at an age of 7–12 months the ratio of glycine to taurine conjugates is 2.4 (3.1 in the adult). In newborns trace amounts of a conjugate of cholic acid presumably with L ornithine has been isolated (49). In livers of immature guinea pigs and rats ornithocholanic acids have been isolated (48).

Bile acid metabolism has been investigated by isotopically labeled cholic acid in normal subjects and in patients with various liver diseases (3 5). Normally less than 0.1 per cent of the isotope is excreted in the urine during the 7 days following the administration of the isotope. Augmentation of the urinary excretion of labeled bile acids is only found in cases of severe hepatic failure.

We have studied the metabolism of intramuscularly administered [14]C labeled cholic acid in 4 cases of intrahepatic cholestasis. The cholic acid was almost exclusively excreted with the urine. No transformation of cholic acid was observed but it is impossible to state if this is due to an interrupted enterohepatic circulation or to the absence of bile acid transforming microorganisms in the intestine of children at these ages. The capacity of the liver cells to conjugate bile acids seemed to be intact in intrahepatic cholestasis of infancy since the bile acids excreted in the urine were almost quantitatively conjugated. In addition to glyco- and taurocholic acid at least three more but not identified conjugates of cholic acid were isolated. As the patients I J and L B had no such conjugates in the gall bladder bile it is likely that these conjugates are predominantly excreted by the urine and not by the bile. It is unknown whether these conjugates occur normally in early infancy or if they appear as a result of the liver disease. These types of conjugates have not been found in studies of ... ad conjugates in gall bladder bile and intestinal contents of children of different ages (8 17 49).

Bile obtained from three of the patients showed a normal appearance due to the excretion of bilirubin but the concentration of bile acids was remarkably low. Since the concentration of bile acids in gall bladder bile has been reported to be low in the first week of life (8 49) it may be speculated whether there is a deficiency of an enzyme system responsible for the transport of bile acids from the liver cells to the bile ducts in newborns. It may then be possible that there is a persistence of or even more likely progression of the deficiency of this enzyme system in intrahepatic cholestasis of infancy. It is remarkable that even when icterus had disappeared in patient I J 35 per cent of the excreted isotope was still eliminated in the urine. However no data are available of the urinary excretion of bile acids in normal infants at different ages.

*Malabsorption*

In all 4 patients there was a moderate to severe steatorrhea. A poor absorption of fatty acids could also be seen from the lack of a rise in the serum triglyceride level after a testmeal with cream. Fat absorption became still more impaired during the relapse in patient L B. Steatorrhea was accompanied by a very low serum concentration of vitamin A in two of the patients (A W and L B). In three of the patients severe impairment of vitamin A absorption was demonstrated. However none of the patients exhibited symptoms of vitamin A deficiency. In spite of the severe longstanding steatorrhea in two of the patients A W and L B there was no clinical or biochemical evidence of rickets. The poor nutritional condition with retardation of growth found in two of the patients (A W I B Fig 1) might well be explained to be the consequence of malabsorption. When steatorrhea diminished in case I J the growth rate became normal.

The results of fat balance studies and studies of bile acid excretion clearly demonstrate a correlation between steatorrhea and impair

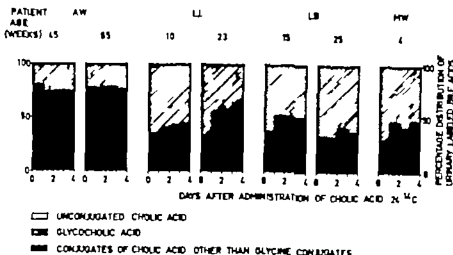



Fig 9 Composition of urinary labeled metabolites

L B ) originally suggested an associated atresia of the bile ducts (39 60) However this possibility was excluded by operative cholangiography In case A W the existence of extra hepatic bile duct atresia was excluded as the result of operative cholangiography and in the fourth case M W by the recovery The results of various laboratory examinations excluded disorders in all 4 patients which may be accompanied by cholestasis in early infancy such as galactosemia (4) hereditary tyrosinemia (6) cystic fibrosis (22, 62) erythroblastosis due to isoimmunization (50 57 67) and infections like toxoplasmosis (42) syphilis (47) cytomegalovirus disease (58) and rubella (59) The mother of the patient A W was jaundiced during the last month of pregnancy The course of her disease followed that of the syndrome of jaundice in pregnancy (cf 1 54) but infectious hepatitis cannot be fully excluded

*Liver function tests*

The clinical course and the results of various liver function tests varied from patient to patient The signs of cholestasis appeared at ages between 10 days and 2 ¹/ months Jaundice had already disappeared in case M W at 6 weeks and also in case I J but not until the baby was 5 months of age In the remaining two patients A W and L B hyperbilirubinemia still persists There was a recurrence after a period of slight jaundice in both the latter patients Hypertriglyceridemia and hypercholesterolemia were only present in patient A W Galactose tolerance was normal in all 4 patients The activity of several enzymes may be elevated during the acute course of the disease as shown by the results of determinations of the serum activities of glutamic oxalacetic and glutamic pyruvate transaminases and of alkaline phosphatases, leucine amino peptidase and $\gamma$ glutamyl transpeptidase However there was no regular pattern neither for the transaminases nor for those enzymes considered to be elevated in blood during cholestasis There was also a lack of correlation between the activities of the various serum enzymes and the concentrations of total and direct reacting bilirubin These results agree with other reports that the activities in blood of alkaline phosphatases and transaminases are poorly correlated to the clinical course of the disease (53 66)

*Bile acids*

The two main bile acids formed from cholesterol in man are cholic and chenodeoxycholic acid Secondary bile acids are formed from these by the action of intestinal microorganisms and absorbed in the intestine The main secondary bile acid formed from cholic acid is deoxycholic acid The proportion between cholic acid and chenodeoxycholic acid in infants 1–7 days old is 2 5 1 (17) but decreases during the first month to the normal adult ratio of about 1 2 1 Infants lack the microbial 7α-

dehydroxylation and deoxycholic acid is rarely detected in children under the age of one year (17, 49). During the first days after birth the bile acids are mainly conjugated with taurine but the proportion of glycine conjugates rapidly increases and at an age of 7–12 months the ratio of glycine to taurine conjugates is 2.4 (3.1 in the adult). In newborns trace amounts of a conjugate of cholic acid, presumably with L-ornithine has been isolated (49). In livers of immature guinea pigs and rats ornithocholanic acids have been isolated (48).

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The results of fat balance studies and studies of bile acid excretion clearly demonstrate a correlation between steatorrhea and impair-

ment of the transport of bile acids to the intestines When the excretion of bile acids to the gut was found to improve as in case I J steatorrhea diminished Absorption of vitamin A was particularly impaired This finding indicates the importance of an adequate intestinal concentration of bile acids for the absorption of this vitamin (cf 30)

As already mentioned, the poor nutritional state in patients with intrahepatic cholestasis is due most likely to malabsorption It may be beneficial to replace saturated fatty acids in the diet by polyunsaturated fatty acids which are more easily absorbed (20) The extent of absorption of medium chain triglycerides (MCT) in conditions with low bile acid concentration in the intestine varies according to different investigators (11 65) Due to this no administration of MCT has yet been tried in our patients Owing to the poor absorption of fat soluble vitamins in intrahepatic cholestasis of infancy it may be necessary to administer them by the parenteral route

The resin cholestyramine has been used to reduce itching in cholestasis However since there is an almost total lack of bile acids in the gut this treatment cannot be expected to cause relief in intrahepatic cholestasis of infancy

*Clinical implications*

There are some clinical implications of the results obtained Since exploratory laparotomy may be harmful for patients with intrahepatic cholestasis (23 32) a laboratory test by means of which a distinction can be made between this condition and bile duct atresia has been greatly searched but no such test has as yet been devised Although tests for the excretion of radioactive rose bengal should theoretically permit differentiation between the complete obstruction of atresia and less complete stasis in the syndrome of intrahepatic cholestasis this does not seem to be the case in practice (25) It is quite evident from the results given here that studies of the excretion of bile acids also do not contribute towards the differentiation between the two conditions In fact in cases of atresia of the bile ducts we have studied (46) the metabolism and excretion of labeled cholic acid was found to be about the same as in cases of intrahepatic cholestasis

The results obtained do not give any information concerning the etiology and pathogenesis of intrahepatic cholestasis of infancy Bile acid excretion to the gut has been found to be severely affected In fact this function of the liver seems to be more disturbed than any of the other functions studied It remains unknown whether the impairment of the excretion of bile acids via the bile is the main cause of the disease or only the consequence of a generalized dysfunction in the excretory capacity of the liver

## SUMMARY

Bile acid excretion has been studied in four patients with intrahepatic cholestasis of infancy (neonatal hepatitis) after intramuscular administration of cholic acid 24 $^{14}$C

Bile acid secretion to the intestines was found to be highly impaired and the main route of excretion was via the urine Practically all of the administered labeled cholic acid was conjugated prior to excretion The main conjugates were glycocholic and taurocholic acid At least three additional conjugates of cholic acid were isolated from the urine

Analysis of bile obtained from three of the patients in connection with operative cholangiography showed a very low concentration of bile acids phospholipids and cholesterol The bile was of normal colour owing to the presence of bilirubin

Severe steatorrhea and markedly impaired absorption of vitamin A was demonstrated when the patients were jaundiced The impairment of bile acid excretion to the gut and the degree of steatorrhea were well correlated In some of the patients steatorrhea persisted after the disappearance of jaundice In those instances the impairment of bile acid excretion to the gut was found to persist

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*Key words* Cholestasis in infancy neonatal hepatitis giant cell hepatitis bile acid excretion bile acid conjugation steatorrhea vitamin A absorption malabsorption

*Acta Pædiat Scand 58 73–79 1969*

CASE REPORT

# ENCEPHALOPATHY IN COMBINATION WITH A NEW PATTERN OF AMINOACIDURIA

T BERGE N BORGFORS A BRUN and W VON STUDNITZ

*From the Departments of Pathology and Clinical Chemistry University of Lund General Hospital Malmo Department of Paediatrics General Hospital Malmo and Department of Pathology University of Lund Lund Sweden*

Many diseases have been found to be associated with amino-aciduria which is often of a primary type and caused by an inherited defect in an enzyme or enzymes mediating the transport or metabolism of the amino-acid(s) The condition is usually accompanied by mental retardation The types of amino-aciduria hitherto described have been surveyed by Efron (5)

Below a report is given of a child with a peculiar clinical picture and a specific amino-aciduria as well as certain neuro-pathological findings This set of symptoms and signs might be a new syndrome

## CASE REPORT

The patient (P 7136/65) was born Oct 30th 1965 five weeks before term The pregnancy was normal Wassermann's reaction was negative No medicines except aminoadiposaines was taken Delivery was uncomplicated and foetal heart sounds were normal during labour No sign of strangulation by the umbilical cord The cord and the placenta (weight 600 g) were both of ordinary gross appearance

The boy appeared normal Weight 3130 g Length 47 cm Skull circumference 34 cm Apgar score 9 (peripheral cyanosis) The mother was 35 years old No abortions Two boys born 1953 and 1962 were healthy No consanguinity No hereditary disorders are known

Six hours after birth the child was transferred to the paediatric department because of tachypnoea (80/min) and persistent peripheral cyanosis Mild general hypotension was noted and spontaneous activity was weak Moro embrace reflex normal Sucking reflex first weak after two days invariably negative Grasp reflex absent on both sides Fontanelles were of normal tension and size Facial features were somewhat coarse During the first 4 days there was an increasing tendency of the head to be drawn backward At the same time jaundice developed On the fifth day of life at a bilirubin level of 21 8 mg/100 ml (Hb 20 0 g/100 ml) exchange transfusion was given with O Rh negative blood without complications The serum bilirubin level after the exchange was 9 1 mg/100 ml Within 2 days the bilirubin again rose to 18 7 mg/100 ml thereafter it decreased to 1 7 mg/100 ml on the 16th day (Hb 15 3 g/100 ml)

From the second week even very gentle handling of the child strongly accentuated the dyspnoea but blood gas analysis and chest X ray was normal A harlequin phenomenon was occasionally seen The peripheral cyanosis persisted unchanged but Apgar score was never below 9 There was only little spontaneous activity The patient was lying in an increasing and finally extreme opisthotonus but had no convulsions The facial features became more and more grotesque (Fig 1) The child was unable to suck and was therefore fed via a stomach tube with breast milk and a formula There was no vomiting The lowest bodyweight was 2640 g By the time of death (38th day) it had recovered the level at birth (3140 g)

The temperature was normal until two days before death when it began to rise The dyspnoea changed the last days into a peculiar pattern with series of long deep breaths directly followed by series of very hasty shallow breaths at about 20 second intervals Blood gas analysis now revealed a moderate acidosis which was readily controlled by bicarbonate infusion The ECG was normal until the last few days when it showed a bizarre pattern with large P waves a QRS-complex varying widely in breadth and amplitude as well as single nodular and ventricular extrasystoles

On the first day of life the only EEG recording taken was abnormal Most of it was distorted by movement artefacts Artefact free segments tended to show periodic high voltage polyphasic complexes

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On the last day of life the only EEG recording taken was abnormal Most of it was distorted by movement artefacts Artefact free segments tended to show periodic high voltage polyphasic complexes



*Fig. 1* The child 19 days old

These bursts lasted between 1 and 2 seconds and tended to recur at 8–10 second intervals The inter burst EEG *showed an irregular low voltage pattern* and in some sequences a nearly isoelectric record

The child died in the type of respiratory distress described above 38 days old

*Clinical and laboratory studies*

Otorhinolaryngological and ophthalmoneurological examinations revealed nothing abnormal X rays of the skull spine and chest no pathological findings Cerebrospinal fluid obtained on the 4th day normal 12th day no cells total protein concentration 60 mg/100 ml 36th day 0 6 polynuclear and 17 mononuclear cells/mm total protein concentration 80 mg/100 ml Subdural punctures on the 4th and 36th day did not reveal any abnormality The skull circumference remained unchanged 34 cm Blood group mother and child O Rh positive Coombs test negative The mother had no Rh – antibodies but a titer anti A 1 128 of so called immune type Direct van den Bergh reaction negative Repeated examination of the blood sugar haemoglobin electrolytes and serum alkaline phosphatase revealed no abnormalities With the exception of slight acidosis the day before death the blood gas analyses were normal Total leucocyte counts and differential counts were normal Guthrie test (phenylketonuria) normal Tryptophan loading test normal

Nasal secretion and throat cultures growth of pyocyaneus coliform rods Klebsiella and proteus *Cerebrospinal fluid* no growth *Culture of faeces for* listeria was negative No viruses could be isolated in cultures of faeces throat swabs blood and cerebrospinal fluid

Serological investigations of the mother influenza A parotitis polio 1–3 and adenovirus normal values Herpes simplex 1/20 Listeria Agglutination 1 256 (1/128 after absorption with staphylococci) and 1/128 Complement fixation 1/10 1 10 and 1/15 Toxoplasmosis Dye test 1/50 and 1/50 Complement fixation 1/15 and 1/15 In view of the listeria titer found in the mother the child was treated with injections of terramycin 20 mg × 2 from the 4th to the 28th day of life For short periods the patient was also given ampicillin and benzylpenicillin

Chromosome studies were not performed

*Autopsy*

A full term baby boy with grotesque facial features but no other external or internal malformations Normal amount of subcutaneous fat

The lungs (right 31 g left 31 g) showed alternating aerated and atelectatic areas with red brown cut surfaces The trachea and bronchi contained a small amount of grey yellow secretion

The liver pancreas kidneys adrenals spleen thymus and thyroid were of normal appearance

Macroscopically the lungs were markedly congested In some areas there were deposits of lymphocytes interstitially There the alveoli contained proteinaic fluid and numerous macrophages in some of which the cytoplasm was vacuolated No substance positive for fat could be demonstrated

The liver kidneys and the other organs mentioned above were of ordinary histologic appearance

*The brain* was of normal gross configuration and weight (430 g) The cut surfaces showed a grey gelatinous white matter with scattered punctate white shiny foci around the lateral ventricles in the frontal and parietal lobes (Fig 2) The spinal cord was macroscopically unremarkable The further studies were performed after fixation of the central nervous system in neutral formalin

A large number of sections of the brain were studied histologically with Nissl's method haematoxylin-eosin Mahon's myelin staining method with acetic acid-cresyl violet for metachromasia and for fat with Sudan red

The brain was of immature appearance with cellular meninges and cortex with some areas of persistent subpial granular layer in the cerebrum and mostly immature nerve cells in the cortex Only in the deeper cortical layers were pyramidal cells seen

The white matter was cellular and contained periventricular remnants of matrix in the form of paravascular cords of undifferentiated cells There was no visible myelin in the cerebrum except in the pyramidal paths in the lower part of the capsula interna and in the peduncles

The cerebral cortex showed a few minute pin shaped areas of reduced stainability and with single fatty macrophages There were however no larger lesions or laminar necroses

The entire white matter was hyperaemic and the perivascular spaces were moderately widened The dominating change consisted of focal lesions most common around the anterior horns of the ventricular

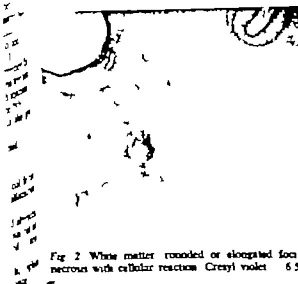


Fig 2 White matter rounded or elongated foci of necrosis with cellular reaction Cresyl violet 6 5

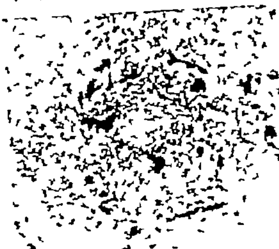


Fig 3 White matter focal lesion surrounded by engorged vessels and glial proliferation forming an indistinct wall Note filamentous concrements at periphery of lesion Center of lesion filled with fatty macrofages Haematoxylin and eosin 100

system sometimes radiating out towards subcortical white matter These changes which corresponded to the gross white shiny lesions were characterized by astrocytosis microglial proliferation increased vascularity and loosening of the ground substance with deposition of large fat-containing macrophages and single roundcell like elements and polymorphonuclear leucocytes as well as some pyknotic forms of cells Some of the foci also contained concrements which were larger than the astrocytes and either rounded and built up of granules coalescing to form an amorphous mass or elongated rods with the appearance of encrusted cell components They were stained basophilic in haematoxylin-eosin Around these focal changes and more diffusely in the white matter gliosis was seen with some areas of fairly abundant large protoplasmic astrocytes with pale nuclei situated polarly and eccentrically in an eosinophilic rounded cytoplasm These cells differed from other astrocytes which had a pale nucleus but only little often elongated cytoplasm (Figs 3 4)

Spongy loose fairly well-defined areas without any definite cellular reaction were also seen mainly subcortically

The cerebellum had a thick outer layer of granular cells The Purkinje cells and inner layer of granular cells were well preserved As in the cerebrum the white matter was markedly hyperaemic but contained no such focal changes

In the spinal cord and the brain-stem the dorsal tracts lemnisci and nerve roots were partly and better myelinated than the lateral tracts and especially than the pyramidal pathways But here no focal pathological changes were seen with certainty

Nowhere in the central nervous system were any deposits of abnormal metabolites or metachromatic



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Table 2. *α Amino nitrogen in urine in mg per gram creatinine*

| Patient | 573–562 |
|---|---|
| Mother | 51 |
| Father | 49 |
| Brother 1 | 101 |
| Brother 2 | 115 |

per 100 ml plasma. Quantitation of various plasma amino acids could not be performed because of insufficient plasma available. Only a chromatographic separation of the plasma amino acids was done for screening purpose. As compared to normal plasma a distinct elevation of the same amino acids as in the urine, i.e. alanine, serine and threonine could be registrated. The spots of these amino acids appeared to be roughly in the same concentration. In addition to these amino acids lysine, glycine, glutamic acid and valine exhibited distinct spots on the chromatogram. Their concentration were estimated to be less than 25 per cent of the above mentioned amino acids.

The plasma-α amino nitrogen in the mother was normal (6.2 mg per 100 ml). The amino acid of the urine in the patient's parents and 2 brothers was normal (Table 2). Amino acid chromatography revealed no abnormality.

The urinary α amino nitrogen of seven premature infants with symptoms of hypoxia was measured by the same method. The values varied between 278 and 450 milligrams per gram creatinine. Chromatography revealed no abnormality in the pattern of the urinary amino acids.

## DISCUSSION

The laboratory investigation of our patient revealed an increased urinary excretion of α amino nitrogen amounting to 573 mg per gram creatinine. Chromatographic separation of the amino acids showed that this was caused by an increased excretion of alanine, serine and threonine. It was also shown that the α amino nitrogen of the plasma was clearly elevated (10.5 mg per 100 ml). This elevation was due to an increase of alanine, serine and threonine as shown by chromatography. Even if no quantitative determination of the various amino acids could be performed this finding suggests strongly that the amino-aciduria was caused by an overflow mechanism and that the increased urinary excretion of these amino acids must be due either to an increased synthesis or to a decreased catabolism.

In this particular case we have no conclusive data arguing for either alternative. No studies have been done to rule out an enzyme defect of the catabolism of the amino acids involved.

To our knowledge no patient has hitherto been described with increase of only serine, alanine and threonine.

Berry (2) found an increased excretion of threonine and serine in a few children with retarded mental and physical development, galactosaemia and some other conditions. She found that alanine excretion is roughly parallel to that of glycine. She also found an increased alanine excretion in a few mentally retarded children in galactosaemia and in some other diseases such as certain types of anaemia combined with hyperglycinuria. Berry (2) gives no detailed account of these patients. She mentions only the possible role of a liver dysfunction in the case with impaired threonine and serine metabolism. Our patient had no liver damage of type seen in galactosaemia. There was only slight stasis and no fatty changes. Neither was galactose found in the urine.

King & Wainer (7) described a 32 year-old woman with cystinuria who had several additional biochemical abnormalities including high plasma levels of serine, threonine and alanine. Those levels were corrected by pyridoxine administration.

Myelinisation and cytoarchitectonic maturity in this case was somewhat retarded for age. It might be of interest to note that in phenylketonuria and Maple syrup urine disease retarded myelinisation was suspected (9, 13). These diseases can however be excluded in



*Fig 4* Diffuse gliosis around lesions Note plump sometimes binuclear astrocytes Haematoxylin and eosin ×208

substances demonstrated Neither were there signs of kernicterus or inflammatory changes suggesting encephalitis or meningitis

## CHEMICAL STUDIES

### *Method*

All urine samples from the patient as well as from the controls were acified (pH 3) and stored at 4 C until the analysis was performed This was done within 3 days

The α amino nitrogen of the free amino acids in urine and plasma was measured according to a method described by Khachadurian *et al* (6) The plasma was filtered over night through a Visking tube with a positive pressure of 0 7 kg per cm The mean values found on analysis of plasma from 10 healthy adults and 6 children aged 0–1 year were 5 8 and 4 9 mg per 100 ml with standard deviations of 1 3 and 1 5 respectively The α amino nitrogen value of the free amino acids in urine obtained from 85 children below 5 months never exceeded 415 mg per g creatinine Samples of urine with 5 mg of creatinine were initially passed through a 3 cm column of ion change resin (Zeo karb 225 in H± form) to remove urea and reduce the volume Amino acids were then separated by high voltage electrophoresis on Whatman No 3 MM paper at pH 1 2 (formate—acetic acid buffer) The voltage gradient was 40 V per cm and the running time 2 hours Further resolution of the amino acids was achieved by ascending paper chromatography (acetic acid n butanol water 1 4 5 v/v, upper phase) in a perpendicular direction Spots located by spraying first with ninhydrin and then with copper reagent, were eluted from filter paper with methanol and the eluate was quantitated spectrophotometrically at 504 mμ Standard amino acid solutions and blanks were run at the same time as each urine sample Because of the difficulties in collecting timed urine samples all values were expressed in terms of milligrams per gram of creatinine measured by the Jaffe reaction Since a considerable hydrolysis of glutamine to glutamic acid takes place on the ion exchange resin the values for both components were added and reported as one value

### *Results*

The α amino nitrogen of the patients urine on the 23rd and 31st day of life was 573 respectively 562 mg per gram creatinine Chromatographic separation of the urinary amino acids revealed besides several amino acids in normal concentrations (glycine histidine methylhistidine lysine glutamine glutamic acid) three main spots which belonged to alanine serine and threonine The excretion of these amino acids was considerably increased about 3–4 times that reported to be normal The values are given in Table 1

The α amino nitrogen of the patient's plasma obtained at the 16th day of life was 10 5 mg

Table 1 *Amino acids in patients urine in mg per gram creatinine*

| | 23rd day | 31st day |
|---|---|---|
| Alanine | 1350 | 1300 |
| Serine | 1150 | 1064 |
| Threonine | 930 | 890 |
| Glycine | 300 | 354 |
| Histidine and methylhistidine | 200 | 124 |
| Lysine | 100 | 75 |
| Glutamine and glutamic acid | 50 | 55 |

The widespread focal lesions in the central nervous system found at autopsy are probably capable of producing a large range of symptoms and to disturb the normal development of a child. We therefore think that they are related to the disorders demonstrated in vivo and that they might be caused by this new type of amino-aciduria.

## SUMMARY

A male infant born 5 weeks before calculated term had tachypnoea and peripheral cyanosis and its spontaneous activity was decreased. No sign of anoxia at delivery. The increased serum bilirubin was treated with exchange transfusion and on the 16th day of life the serum bilirubin level was normal. The child gradually developed signs of injury to the central nervous system and amino-aciduria involving serine, alanine and threonine was discovered. The same amino acids were also found in increased concentrations in the serum.

The child died at 38 days of age. Autopsy revealed multiple focal changes in the cerebral hemispheres. The changes consisted of focal necroses of white matter with gliosis and deposition of fat-containing macrophages. The changes were of the same type as those described previously in certain inborn errors of metabolism but showed no specific features. Similar changes may also be seen in hypoxia but in the present case there was no evidence for such an origin. No specific amino-aciduria could be demonstrated at examination of 7 premature children with hypoxia. The kidneys showed no histological abnormalities and the fact that the concentration of the amino acids was increased both in the serum and in the urine argues against a renal origin.

No explanation for the aetiology could be offered but it was probably due to an inborn enzyme defect.

## ACKNOWLEDGEMENT

We wish to thank I. Sulg, M.D., for help with the interpretation of the EEG. In part supported by Statens Medicinska Forskningsråd. Grant no. B68-12X-2037-02.

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*Key words:* Aminoaciduria, encephalopathy, alanine, serine, threonine

the present case In these and other forms of amino aciduria cerebral changes have been described in the form of oedema vacuolisation and swelling of myeline sheaths or focal demyelinisation with scar tissue formation and a diffuse spongy loosening or spongy degeneration (4 9) In the present case only small areas of spongy appearance without glial proliferation were seen subcortically, but no clearcut brain oedema The changes in the myelin sheats could not be evaluated because of the scantiness of the myelinisation In view of the increased titer for listeria in the mother the possibility of listeriosis was also considered The paraventricular cerebral lesions however lacked the granulomatous necrotic character which is characteristic of listerial lesions and the eosinophilic concrements which have been described in this condition No granulomatous changes could be demonstrated in the meninges or any of the other organs studied histologically

*The focal cerebral lesions resemble* changes which have long been known particularly in prematures and by Banker *et al* (1) called *periventricular leucomalacia of infancy* which they ascribed to ischaemia anoxia A large number of their cases had episodes of apnoea *cyanosis cardiac arrest or cardiovascular mal* formations An anoxic ischaemic aetiology of the focal changes in the present case cannot be excluded with absolute certainty on a morphological basis but there were no additional cerebral lesions of the type seen in hypoxia (e g laminar necrosis) From a histopathological point of view the lesions described may well have developed during postnatal life The clinical picture during labour and the neonatal period was not that of serious hypoxia It was not possible to reveal the cause of the tachypnoea or the final peculiar respiratory pattern by standard clinical investigations *Anyhow he* had only slight peripheral cyanosis and had always had a high Apgar ascore (never below 9) and *no cardiovascular* changes That amino-aciduria involving only serine, threonine and alanine should be secondary to hypoxia appears less likely and no amino-aciduria could be demonstrated in 7 premature children with hypoxia

The focal changes also *resemble those* described by Malamud (9) and demyelinisation foci described in phenylketonuria by authors cited by him In the absence of clinical and pathological support for an anoxic ischaemic etiology one should thus consider the possibility of a relationship with the patient s metabolic disorders in analogy with what is seen in phenylketonuria and some of the other types of amino aciduria mentioned above

In other examples of amino aciduria cerebral changes varied from none or inconsistent to cortical degeneration and severe malformation (10) More often however the cerebral investigation was not reported or if so only briefly Consequently the neuro pathology of amino-aciduria is not properly known

The EEG-pattern with repetitive polyphasic bursts is reminiscent of the abnormalities described in subacute sclerosing *leukoencephalitis* (12) and cerebral lipidosis (3)

Pampiglione (11) reviewed EEG abnormal*ities in inborn errors of metabolism* but reported no observations pertinent to amino aciduria

Lesse *et al* (8) studied 12 patients with severe diffuse encephalopathy manifested by organic mental impairment dyskinesia rigidity myoclonus and generalized seizures The EEG showed bilaterally synchronous slow bursts that recurred periodically at 5 to 10 second intervals They inferred that EEG patterns might be similar despite diffrences in aetiology Because the EEG was recorded only once in the present case further speculation appears unjustified

Characteristic of the *child* were *tachypnoea* a final respiratory pattern with series of long deep breaths directly followed by series of very *hasty shallow breaths at about* 20 second intervals EEG abnormalities, peripheral cyanosis opistotonus stationary circumference of the skull inability to suck poor increase in weight and increasingly bizarre facies all of which were interpreted as a possible effect of injury of the central nervous system



*Fig. 1.* Interstitial fibrosis and lymphocytic infiltration, scarred glomeruli partly cystic. Arrow indicates deposit of hemosiderin. Hematoxylin-eosin. ×237 (Ivemark.)

DISCUSSION

The pathogenesis of renal vein thrombosis does not differ from that of thrombotic diseases in general. Changes in the renal blood flow and pathologic processes in or around the renal vessels are significant. The fact that the disease is more frequent in the neonatal period than later in childhood points to predisposing factors in this age group. One may be the high viscosity of the blood due to the high hematocrit value, which is particularly high at dehydration, at intrauterine transfusion in twins and at diabetic foetopathy. In infants with very high hematocrit values there is a risk of cardiac failure but also of erythrocyte sludging in the kidney. A slow blood flow influences the equilibrium of pro- and anticoagulant principles in the vascular periphery and may result in a hypercoagulability (4).

Microscopic examination of kidneys subjected to vein thrombosis has revealed thrombotic processes intrarenally or in the large renal veins (9). In some cases there has been a recanalisation, in others there has been a cortical necrosis without any signs of thrombosis. In these cases fibrinolysis of the thrombi may have occurred before the examination or the fibrin deposits may have been submicroscopically visualized at first with electrone microscope (4). In one case ending in death eight hours after the clinical debute there was only a vasodilatation in the renal vessels (2).

Renal vein thrombosis has been considered as an extremely serious condition which requires prompt surgical intervention (6, 8). In the last years, however, some cases with benign course have been reported (2, 3, 5). The clinical manifestations appear a few days after the birth. The typical features are signs of dehydration, hematuria and an abdominal mass in the flank. The blood pressure usually is normal, sometimes there is discrete edema. The general condition is rather unaffected. Intravenous urography may reveal a non-functioning kidney. Thrombocytopenia may occur. The flank mass continuously diminishes during 2–3 weeks but the hematuria may remain any longer. The infants seem to recover but an intravenous urography several months later has still shown a non-functioning kidney. Michon and Gaillard *et al.* (3, 5) have reported three cases of bilateral enlarged kidneys and hematuria which all have recovered. Two of them were treated with heparin and they had a normal urography 2–5 months later.

If an infant of a diabetic mother is plethoric

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CASE REPORT

# RENAL VEIN THROMBOSIS IN A NEWBORN INFANT OF A DIABETIC MOTHER

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Vein thrombosis in infants has been observed in connection with dehydration with severe infectious diseases and following complicated deliveries Usually the renal and suprarenal veins are affected and most cases occur in the neonatal period Renal vein thrombosis in an infant of a diabetic mother was first reported in 1957 by Avery *et al* (1) From autopsy findings it is evident that renal vein thrombosis particularly occurs in newborn infants of diabetic mothers (7) The following case illustrates some of the problems which are encountered in the treatment of renal vein thrombosis in a newborn infant

## CASE REPORT

The patient a newborn girl was the second child of a 27 year old woman with diabetes since 17 years of age The mothers diabetes had been well controlled except for moderate glucosuria during both pregnancies The first pregnancy two years earlier had ended in a stillborn infant with features typical of diabetic foetopathy

The mother was admitted to the obstetric clinic seven weeks before term for control of her diabetes The following two weeks she had glucosuria of 50–100 g per day but later her disease was kept under adequate control At the 36th week of gestation a cesarean section was performed without complications The infant weighed 3 6 kg She had abundant subcutaneous fat and a puffy plethoric face The hematocrit value was 72 On the second day of life the baby had macroscopic hematuria and two days later there was a palpable mass in the left flank The systolic blood pressure increased to 110 mm Hg Blood urea nitrogen was 12 8 mg per 100 ml and the Quick index was 50 There was no oliguria but on intravenous urography no contrast excretion could be seen from the left kidney The general condition was unaffected there was no edema and the appetite was good Weight gain was normal The hematuria disappeared after a week The mass in the flank diminished in size and was not palpable 14 days later when the patient was discharged from the hospital

The baby developed well but after four months the left kidney was still non functioning at intravenous urography and the blood pressure was elevated to 125 mm Hg systolic In the electrocardiogram there were signs of left heart hypertrophy The urinary excretion of aldosteron was high 3 6 μg/24 hrs Because of the progressive hypertension and since the left kidney did not function nephrectomy was performed Before the operation catheters were put up in the ureters and the urinary flow was measured during mannitol diuresis for seven hours From the left ureter only 1 ml urine was collected but from the right 60 ml At operation the left kidney was removed It looked macroscopically normal and so did the renal vein Postoperatively the blood pressure rose to 220 mm Hg systolic but after four days it was normalized For the rest the postoperative course was uneventful and the patient was discharged one month after the operation

Histology of the removed kidney showed considerable deposition of hemosiderin and atrophy of the renal cortex (Fig 1) The parenchyma was damaged in great areas with proliferation of Bowman's capsule hyalinized glomeruli and interstitial fibrosis Any thrombi could not be pointed out but the changes corresponded with those seen at thrombosis of the renal vein (Ivemark)

After the operation the girl has been followed by clinical examinations During her first year of life she has developed normally but she has had three urinary tract infections At the last control her blood pressure and blood urea nitrogen were normal

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CASE REPORT

# DEFIBRINATION SYNDROME IN A NEWBORN AND ITS TREATMENT WITH EXCHANGE TRANSFUSION

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In children the defibrination syndrome is rare and has been described in connection with purpura fulminans (3 4) hemolytic uremic syndrome (5) and thrombotic thrombocytopenic purpura (6) The purpose of the present study is to describe a defibrination syndrome in a *newborn* boy and its successful treatment with exchange transfusion

## CASE HISTORY

Our patient was the second child of healthy parents born July 29 1966 at term in breech presentation He was severely asphyxiated Intubation and aspiration of the airways were immediately done After some minutes of artificial respiration he started to breathe spontaneously but with grunting respiration One mg of phytomenadione was given i m About 5 / hours after birth he was found lying in opisthotonous position with maximal plantar flexion of his toes The muscles were generally spastic Petechiae were observed in the neck and inguinal regions *P & P* was 17* and 0.5 mg of phytomenadione was given He was then transferred to the Department of Pediatrics Rikshospitalet Oslo

On admission he was 9 hours old severely ill nearly without any spontaneous movements He was in a shocklike condition the skin was grayish pale cold with several petechial hemorrhages The umbilical cord central to the clamp was distended with blood The body temperature was 35.8 C the pulse rate 120 per minute The fontanel was bulging The eyes were closed and bulb deviation to the right was observed The pupiles were equal gave normal response to light and the eyegrounds were normal Spasticity in the lower extremities with plantar flexion of the toes was present and the deep tendon reflexes were increased The Moro reflex was nearly absent. The cerebrospinal fluid contained visible blood

During the next hours the condition deteriorated In the palms of the hands and the soles of the feet with a sharp border to the normal skin on the dorsal side the skin became deeply blue

The child revealed no clinical signs of infection Specimens from throat nose and blood did not contain bacteria In specimens of blood CSF faeces urine and throat there was no growth of virus Dye test was negative There were no signs of renal damage or pulmonary disease and no signs of cavernous haemangioma Increased hemolysis was not demonstrable The Coombs test was negative

The family history revealed no information of bleeding tendency The mother was healthy during pregnancy and she was regularly controlled She had no signs or symptoms of infection or toxemia and no signs of diabetes She had normal bleeding time and whole blood coagulation time her platelet count was 193 000 on the day of delivery About 1 / hours before delivery she got 75 mg of pethidine cloride s c otherwise no drugs

Laboratory data from the infant on admission (Table 1) showed a marked reduction in fibrinogen and platelets greatly prolonged primary bleeding time and prolonged cephalin time

## THERAPY AND CLINICAL COURSE

The hemostatic defects in this patient suggested a defibrination syndrome He was treated with one exchange transfusion of 500 ml heparinized blood (containing 2500 I U of heparin) During the first 24 hours 40 mg of Actocortin was given i v the next day 30 mg and the steroid therapy was discontinued on the 8th day Penicillin 100 000 I U was given at the end of the exchange transfusion On the third day 20 mg of phenobarbital was given because of generalized convulsions of short duration

with a high hematocrit value a venesectio may ameliorate the patient, especially if there are also signs of cardiac failure If the infant gets enlarged kidney and hematuria the fluid ba lance should be closely supervised and dehydra tion immediately corrected Treatment with he parin may be of value if there are no signs of renal failure Nephrectomy should be con sidered in the acute phase but bearing the spontaneous recovery in mind expectance may be indicated Later the blood pressure the kid ney function and the urography must be fol lowed A nephrotic syndrome and an arterial hypertension may develop or infections may take place in a non functioning kidney Ne phrectomy therefore may be necessary some months after the acute phase


## SUMMARY

A newborn infant of a diabetic mother is de scribed suffering from a renal vein thrombosis with hematuria and a palpable mass in the flank The general condition was unaffected and the symptoms disappeared after two weeks Nephrectomy was performed after four months because of an arterial hypertension and a patho logic intravenous urography

In these cases a high hematocrit value and a hypercoagulability may play a great part of the pathogenesis Therefore venesectio and treatment with heparin may be of value


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*Key words* Renal vein thrombosis infant diabetic mother

appeared within 3 days.—The EEG was first recorded on the 20th day. A slight reduction of the amplitude and frequency was seen. The EEG became normal within 6 months. The xanthocromia and the protein content of the cerebrospinal fluid diminished gradually and the CSF was normal after 4 weeks.

The subsequent course has been normal. When last seen he was 1 year old. He could then speak some single words, his movements were well co-ordinated and he could stand without support. An inconstant strabism of his left eye was considered as a family trait.

## DISCUSSION

When first seen the infant had a severe generalized bleeding tendency which was found to be caused by a defibrination syndrome. This acquired bleeding tendency is usually seen secondary to infections, malignancies or great haemangiomas, but in our case the trigger mechanism might have been the asphyxia with possible aspiration of amniotic fluid. It cannot be excluded that a bleeding tendency might have been present already in foetal life causing CNS hemorrhage and apnoea. The report of purpura fulminans in a newborn (4) indicates that the condition can occur in foetal life without any demonstrable underlying cause. We did not find any signs of hemolytic anemia which should be expected in thrombotic thrombocytopenic purpura.

Heparin is considered to be the drug of choice in the treatment of the defibrination syndrome (1), but the bleeding in the central nervous system contraindicated the use of heparin in full dosage in this case. *Exchange transfusion* using heparinized blood was chosen as treatment because we wanted to supply platelets, clotting factors, a small amount of heparin and possibly remove circulating clot promoting substances and split products of fibrin/fibrinogen.

Steroids were given because of the possibility of adrenal cortical insufficiency and because of the possibility of an immunological mechanism transmitted from the mother.

The fibrinolytic system was not studied. Its pathophysiological role in these conditions is uncertain; most probably fibrinolytic phenomena are secondary to intravascular clotting. The amount of blood available was not sufficiently great for a thorough examination of the fibrinolytic system.


## SUMMARY

A male infant was born asphyxiated, but after few minutes of artificial respiration spontaneous breathing was established. During the next few hours he developed a severe bleeding tendency with signs of intracranial hemorrhage. In the palms of the hands and soles of the feet there were symmetrical large ecchymoses with a sharp border to normal skin. Coagulation studies showed the characteristics of a defibrination syndrome. He was treated with one exchange transfusion of heparinised blood and corticosteroids for one week. After the exchange transfusion a rapid and marked improvement was noted. He was followed up during his first year of life, and the development has been quite normal. No permanent disabilities have been found. It is possible that asphyxia was the trigger mechanism for the defibrination syndrome in this case.

Early recognition of the acute defibrination syndrome and immediate start of therapy may be lifesaving.


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Table 1 *Laboratory data*

Methods for coagulation tests see Egeberg (2) Citrated venous blood was immediately cooled on ice and citrated plasma tested at once Capillary blood was used for determination of hemoglobin and platelets

| | Normal values | Age | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 9 hours | 28 hours | 3 days | 7 days | 20 days | 5 months | 7 months |
| Platelet count (per mm³) | 100–300 ( 10³) | 49 000 | 91 000 | 110 000 | 204 000 | 320 000 | 300 000 | 205 000 |
| Fibrinogen (mg/100 ml) | 180–300 | 17 | 135 | 153 | | 277 | 256 | |
| Quick time (sec) | | 180 | 15 0 – 14 8 | | | 12 8 – 13 2 | | |
| Factor V ( ) | | 52 | > 100 | | | | | |
| PP ( ) | | 29 | 44 | | | 48 | | |
| Bleeding time (Duke) (min) | 5 min | > 30 | | | | 3 | | |
| Cephalin time (sec) | | 135 0 – 118 8 | 52 0 – 53 6 | | | 64 2 – 64 4 | | |
| Hemoglobin (g/100 ml) | 18–22 | 16 3 | 17 5 | 17 5 | | 12 3 | 14 6 | 14 6 |
| Urea nitrogen (mg/100 ml) | 10–50 | 47 | 47 | 80 | | 33 | | |
| Creatinin (mg/100 ml) | 0 4–1 4 | | 1 5 | 0 9 | | | | |

Following the exchange transfusion the condition improved rapidly He recovered from the shocklike condition There were no signs of fresh bleeding and the signs of CNS irritability were markedly reduced He took his first meal at the age of 24 hours and there were later on no difficulties in feeding The signs of increased intercranial pressure vanished gradually The petechiae disappeared within 10 days while the ecchymoses in the palms were still visible the 10th day (Fig 1)

Normalization of the laboratory findings soon occurred The coagulation factors were normal from the second day (Table 1) The first urine specimen was discharged and investigated about 24 hours after birth Mild albuminuria dis



*Fig 1* Ecchymoses in palms Picture taken on the 10th day Note sharp border to normal skin

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Early recognition of the acute defibrination syndrome and immediate start of therapy may be lifesaving.

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CASE REPORT

# RAPIDLY DEVELOPING CONSTRICTIVE PERICARDITIS IN AN INFANT SIMULATING MITRAL VALVE OBSTRUCTION

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*From the Heart Institute Tel Hashomer Government Hospital University of Tel Aviv Medical School and Department of Pediatrics Government Hospital Hadera Israel*

Constrictive pericarditis rarely occurs in early childhood (2 3 6 7 11 15) to the best of our knowledge no proven case of this disease has been described under the age of two years Several authors have stressed the rapidity with which the constriction sometimes develops both in adults (4) and in children (3 7)

Rapidly developing pericardial constriction should be suspected when clinical evidence of impaired ventricular filling such as neck vein distension and hepatomegaly persists while the heart size returns toward normal (1) However the diagnosis of rapidly developing constrictive pericarditis can sometimes be extremely difficult (3 9 11 17) especially in early childhood

This report is of a case of constrictive pericarditis in a 9 month old girl which developed only a few weeks after the onset of acute pericarditis with effusion A special point of interest in this case is that the constriction affected principally the left ventricle The main anatomic basis for this constriction was a large pericardial cyst which gave rise to a misleading clinical hemodynamic and angiocardiographic picture simulating mitral valve obstruction

## CASE REPORT

A 9 month-old girl was referred to the Tel Hashomer Hospital for investigation after having been in congestive heart failure for three months She had been hospitalized the first time at the age of one month with a clinical picture of acute gastroenteritis The stool culture produced Coli 0... After an appropriate treatment with antibiotic and fluid replacement she was discharged in good condition A chest roentgenogram performed during that hospitalization showed the heart and lungs to be within normal limits

At the age of 5 months she was hospitalized in the Government Hospital of Hadera because of fever shortness of breath and coughing On admission she appeared restless respiration 64 per minute heart rate 160 per minute rectal temperature 38 2 C The lungs were clear to percussion but the breath sounds were diminished over the left lower lung The heart sounds were normal and no friction rub was detected The liver was palpated 5 cm below the costal margin The remainder of the physical examination was normal

The results of laboratory investigations on admission were as follows Urinalysis normal hemoglobin 9 g/100 ml white blood count 16 000 mm with 72% neutrophils 27% lymphocytes 1% monocytes serum non protein nitrogen cephalin flocculation thymol turbidity and transaminase were all normal serum sodium concentration was 130 mEq/l potassium 6.3 mEq l and chloride 96 mEq/l

The chest roentgenogram taken on admission (Fig 1) showed normally translucent lung fields and there was moderate generalized enlargement of the heart

The electrocardiogram taken after admission confirmed sinus tachycardia the *P* waves were normal the *P–R* interval was 0 09 sec and the mean manifest electrical QRS axis in the frontal plane was +120 The *T* waves were flat in the right precordial leads

The electrocardiogram taken a week after admission showed development of abnormal *T* waves and ST segments consistent with pericarditis (16)

A repeated chest roentgenogram performed 8 days after admission showed that the cardiac silhouette

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*Key words* Defibrination syndrome newborn exchange transfusion




*Fig 3* Necropsy specimen *LV* Left ventricle *LA* left atrium *P* pericardium *C* cyst in pericardium Note the thickened pericardium over the left ventricle (about 6 mm thick) The pericardial fragment containing the cyst (cyst length 20 mm) is shown separately (*below*) Note the normal structure of the mitral valve

(Fig 3 below) The mitral valve was normal Microscopically portions of the pericardium showed acute subacute and chronic non specific pericarditis There were numerous polymorphonuclear leukocytes and plasma cells with many mononuclear cells some of them with a foamy vacuolated cytoplasma

## DISCUSSION

Acute non sp cific p ricarditis is usually a benign disease As described recently (10) the patient may be asymtomatic throughout the course of the disease despite gross objective findings Pericardial constriction may occasionally appear in this disease (3 4 8 12 3 14 15) and in some instances the pericardial constriction may develop rapidly (3 4 7)

Constrictive pericarditis in early childhood is a rare phenomenon Caddell *et al* (3) reviewed the literature and discovered only 25 cases of constrictive pericarditis in children under 10 years of age in most of these cases the etiology was not established Nadas (11) reported one child aged 2 years with constrictive pericarditis caused by tuberculosis A rapidly developing constrictive pericarditis in a 4-year-old child probably due to Coxsackie B5 virus was described by Gibbons *et al* (7)

Cases of acute pericarditis should be suspected as having developed rapid constriction



*Fig 1* Chest roentgenogram on admission

had widened appreciably Repeated complement fixation tests for viruses were negative

Celbenin 500 mg was given daily in addition to digitalis and steroid therapy for four weeks during which time the temperature fell to normal while the signs of congestive heart failure persisted After failure to improve over another 3 month period she was transferred to Tel Hashomer Hospital for further investigations The electrocardiogram at that time was indicative of marked left atrial hypertrophy and right ventricular hypertrophy was suspected as well The chest roentgenogram showed that the heart silhouette was enlarged Marked pulmonary venous congestion was noted

Cardiac catheterization revealed that the mean right atrial pressure (16 mm Hg) the right ventricular end diastolic pressure (17 mm Hg) and the pulmonary artery end diastolic pressure (18 mm Hg) were all elevated to a similar degree (Fig 2) The right ventricular pressure curve (Fig 2 middle) was also characteristic of constrictive pericarditis showing an early diastolic dip followed by a plateau This plateau was very short because of the rapid heart rate The left atrial pressure curve (Fig 2 right) was markedly elevated (mean 22 mm Hg) with very wide and tall A waves (27 mm Hg)

Contrast medium was injected into the right ventricle and simultaneously antero posterior and lateral angiocardiograms were performed The angiocardiograms showed that the cavity of the right ventricle and the interventricular septum were displaced upwards and to the right The left atrium was distended its volume changes during systole and diastole were minimal while its opacification time was markedly prolonged The left ventricular cavity was in a higher position than normally The infant died a week after the cardiac catheterization in severe respiratory distress and fever due to acute bilateral pneumonia

*At necropsy* the pericardium was found to be diffusely thickened and rigid especially over the left ventricle (Fig 3) where it was approximately 6 mm thick At this site there was a pericardial cyst (2 cm in length) which contained yellow amorphous material

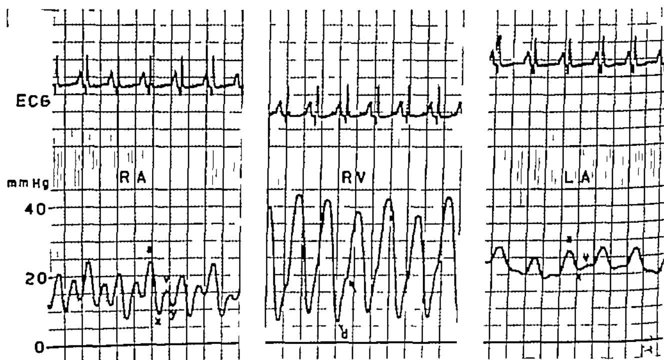



*Fig 2* Intracardiac pressure tracing The right ventricular (*RV*) pressure curve shows an early diastolic dip (*d*) with elevated end-diastolic pressure (17 mm Hg) (*arrow*) The mean right atrial (*RA*) pressure is similarly elevated to the end diastolic right ventricular pressure The left atrial (*LA*) pressure curve is markedly elevated (mean 22 mm Hg) with a very wide tall a wave (27 mm Hg) indicating impeded left atrial emptying




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Cases of acute pericarditis should be suspected as having developed rapid constriction

if the patient's general condition improves, signs of acute infection disappear and the heart size returns toward normal, while evidence of impaired ventricular filling persists (11) The importance of establishing this diagnosis needs no stressing especially in infants as constrictive pericarditis can be successfully operated on at all ages with complete hemodynamic relief (5)

Pitfalls in the diagnosis of the reported case were

1 The fact that the heart size, although decreased in comparison to the acute stage still remained enlarged
2 The electrocardiogram showing development of marked left atrial hypertrophy and some right ventricular hypertrophy
3 Roentgenographic evidence of marked pulmonary venous congestion
4 Angiocardiographic picture of displacement of the heart chambers and delayed left atrial emptying

These findings were fully clarified by the necropsy results The pericardial cyst was acting as a space occupying lesion and partially obstructing the left ventricular cavity mimicing mitral valve obstruction Thus pericardial cyst together with the large left atrium contributed to the enlargement of the heart silhouette which failed to decrease as expected during the development of the pericardial constriction The etiology of the pericarditis in this case was undetermined

## SUMMARY

A case is reported of rapidly developing constrictive *pericarditis in a 9-month-old infant* apparently the youngest yet described

Unusual features of this case were the failure of the heart size to decrease as expected and electrocardiographic roentgenographic angiocardiographic and hemodynamic data which indicated mitral valve obstruction These unusual features resulted from the peculiar location of the pericardial constriction over the left ventricle which took the form of a pericardial cyst hampering left atrial emptying

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# PROCEEDINGS OF PEDIATRIC SOCIETIES

## NORWEGIAN PEDIATRIC SOCIETY

Meeting June 6 and 7 1968

S E Hoyer *Glutenintolerance Introduction to a discussion*

It was suggested that flour products for infants should be made on a gluten free basis because of the frequency and seriousness of coeliac di sease The onset of the disea e could thus be postponed till the child had presumably more resistance

The suggestion led to discussion but the memb rs did not support the idea

Alfred Sundal *Intrauterine growth and neonatal patology*

The rate of stillbirths and prematurity and in consequence the p rinatal mortality might be reduc d by hygienic measures during pregnancy especially with reference to adequate nutrition The perinatal mortality rate in Norway in 1965 was 21 per thousand births as compared to 38 5 in 1935–40 (stillbirths and death in the first week of life)

The material consists of all newborns with a birth weight less than 2500 g admitted to the Children s Hospital in Bergen during the period from November 1965 to April 1968 Their birth weight have been plotted in relation to their gestational age and symptoms of neonatal dis-orders (e g hypoglycaemia respiratory distress acidosis and convulsions) A total of 132 new borns were examined 26 of the newborns were twins or triplets 17 were newborns of toxaemic mothers whereas the pregnancy had been un eventful in 89 cases The birth weight in relation to gestational age gives a better evaluation of a newborn than the birth weight alone Small for dates babies were mainly born to toxaemic mothers (with albuminuria and/or hypertension) Hypoglycaemia ('true blood sugar less than 20 mg p r 100 ml) and symptoms of impaired pulmonary ventilation (cyanosis apnoeic spells) tended to occur in the infants with low birth weight in relation to gestational age and these infants also tended to display vague symptoms of reduced vitality

P E Waaler O Garatun Tjeldsto & P J Moe *Glycogen storage disease*

Little seems to be known about the frequency of glycogen storage disease In Sweden Öcker man found a minimum incidence of 1 246 000 live births Our studies have so far disclosed 14 cases of definite or probable glycogen storage disease in Norway in patients born in the period 1948–1967 This means an incidence of 1 90 000 live births The real frequency is probably higher It is now possible to study the frequency and the hereditary transmission of this disorder by examining the enzyme activity and the glycogen conc ntration in erythrocytes and leukocytes Previous studies of the parents of some patients with type III glocygen storage disease have re vealed intermediary values in the red blood cells indicating that the parents are heterozygotes (van Hoof) The enzyme activity (amylo-1 6-glucosidase measured by the reversible incorpo-ration of glucose $^{14}$C into glycogen) of the red blood cells from members of the family of one of our patients with established type III glycogen storage disease is reported The parents and a brother of the mother appeared to be heterozy gotes while normal values were found in some other family members The results indicate re cessive inheritance of type III glocygen storage

disease The patient's sister and a brother of the mother showed high enzyme activities The significance of these latter observations are discussed It is concluded that further studies are necessary in order to understand the mechanisms of the reported results

S J Sorland *Atrial septostomy in infants with transposition of the great vessels*

The mortality of transposition is high in early infancy When the communication between the two parallell circulations is inadequate palliation can be achieved by resection of the atrial septum But the operative risk is fairly high Rashkind recently used a catheter with a balloon on the tip introduced from the femoral vein via the foramen ovale into the left atrium Partly filled with diluted contrast solution the balloon was repeatedly pulled through the foramen ovale with stepwise increased balloon diameter Cyanosis and congestive heart failure was improved in the 5 infants treated by the author 4 of them neonates one being moribund In one resection of the atrial septum had to be done later Another infant with a very complex heart malformation had severe general retardation and osteosclerosis although he had no congestive failure and only a moderate cyanosis The importance of early diagnosis and treatment is stressed

S E Hoyer *Exceptional vitamin requirement*

The role of individual vitamins in brain metabolism was pointed out Exceptional vitamin requirement was demonstrated in two patients one infant with possible pyridoxin dependency and a two year old boy with metachromatic leucodystrophy who had an acute attack after unintentional ingestion of a massive dose of vitamin A

S E Hoyer *A family with pseudo hypoparathyroidism*

A family of which 4 members definitely have pseudo hypoparathyroidism was described The disease was diagnosed in the mother her two daughters and one son who also had diabetes mellitus In addition another daughter died in infancy almost certainly of tetany while one daughter is healthy

The family history strongly indicates hereditary transmission on the mother's side with females predominantly affected Treatment of two children with calciferol has been started resulting in normal levels of calcium and phosphate One can hardly expect to influence the mental changes

H Pande *Thyrocalcitonin*

Several cases of pseudohypoparathyroidism with very high values of thyrocalcitonin (TC) in the thyroid have been reported lately

Two cases of pseudohypoparathyroidism (mother and daughter) where biopsies from the thyroid gland were examined for TC are reported Both had the typical signs of the syndrome but were *not mentally retarded* The mother was normocalcemic following therapy with vitamin $D_2$ when the test was done Neither of the two biopsies showed highly increased contents of TC The child had a somewhat higher content than the mother but not more than 2-3 times the normal controls

We believe that the high TC content in such cases reported by others is due to and not the cause of the usually accompanying hypocalcemia This theory is supported by the findings of Gittes and Toverud hypocalcemic rats had high levels of TC in their thyroids

O B Schjetne *Cerebral gigantism*

Two children with gigantism were reported The major characteristics of the syndrome were reviewed mental retardation accelerated growth advanced bone age macrocrania and characteristic facies

The two children had the typical general appearance were retarded and had pneumoencephalograms showing dilated ventricles Insulin tolerance seemed decreased in both cases The blood sugar levels after a glucose load showed prediabetic values Growth hormone was found in serum but the response to insulin was abnormal

*Thor Öisten Endsjo*

Acta Pædiat Scand 58 93–96 1969

# PROCEEDINGS OF PEDIATRIC SOCIETIES

## DANISH PEDIATRIC SOCIETY

### Meeting March 13 1968

Oluf Andersen *Demonstration of a patient with presumed osteomyelitis o the neck of the femur*

J Ramsøe Jacobs n *Ami riptyline poisoning Se vere cardiac arrythmia treated with propranolol*
A boy aged three years and weighing 15 kg was admitted approximately five hours after having ingested 700–750 mg amitriptyline (Saroten®) He had generalized seizures which ceased after intra venous administration of 45 mg membutnal sodium He was subsequently deeply unconscious with insufficient respiration and circulation ECG showed severe tachycardia with QRS complexes of constantly varying breadth After intubation and ventilation with oxygen an attempt was made to treat the arrythmia with antazoline electrocon version and with pyridostigmine without result Intravenous administration of 1 mg of the b ta receptor blocking antiadrenergic preparation propranolol immediately converted the arrythmia to sinus rhythm and the bundle branch block di minished The blood pressure became normal The same effect was obtained on subsequent recur rence The patient was unconscious for 36 hours and was slightly hyperexcitable and ataxic during th waking phase The EEG showed moderate non specific changes which had disappeared on *follow up control two weeks later The patient was* clinically entirely symptom free after 4–5 days

Imipramine amitriptyline and relat d prepara tions are employed to a greatly increasing ext nt Cases of poisoning in children are not un com monly reported and have proved fatal in a number of cases Severe cases are charact riz d by gen eralized seizures and circulatory and respiratory insuffi iency The circulatory insuffi iency is due to severe tachycardial arrythmias with varying and prolonged intraventricular transmission of the im pulses The seizures can be controlled by means of barbiturates but these may however caus further deterioration of the respiratory insuf ficiency and also with paraldehyde and chloral hydrate The arrythmias have proved difficult to treat but several communications have been published concerning the effect of pyridostigmine and a few on the effects of lidocaine and propranolol Meticulous supervision is essential for s veral days after successful initial treatment as the serious manifestations of intoxication may r cur

*Discussion*

*E Thamdrup* Emphasized once again the significance of prophylaxis of accidental poisoning and demonstrated a special closing mechanism of Canadian origin for the closure of medicine bottles

*J Øster* Amitriptyline and imipramine are re lated Have cases of poisoning been reported after imipramine?

*O Steincke* In the Christmas Seal Home 125 children have been treated with imipramine with out any cases of poisoning being observed

O Stein ke *Neurofibromatosis Report of a case*

After a brief review of the symptoms of neuro fibromatosis the case of a girl now aged 5 1/ years was presented From the age of one month numerous cafe au lait coloured patches had been observed on the skin of the trunk and extremities At the age of 3 1/ years the child developed a large and persistent swelling on the left side of the neck This was first considered to be lym phadenitis and the patient was submitted to ade notomy during the first admission Six months

disease The patient s sister and a brother of the mother showed high enzyme activities The significance of these latter observations are discussed It is concluded that further studies are necessary in order to understand the mechanisms of the reported results

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*Thor Östen Endsjo*

*Acta Pædiat Scand* 58 97–102 1969

# PROCEEDINGS OF PEDIATRIC SOCIETIES

## SWEDISH PEDIATRIC SOCIETY

### Meeting Oct 21 1967

B Kjellman *Lung clearance index in healthy children*

Lung clearance index (LCI Becklake index) was determined in 36 healthy children 7–13 years of age The equipment and technique described by Svanberg (1) was used

Analyses of the pattern of respiration during the $N_2$ wash-out procedure revealed a certain amount of hyperventilation which did not decrease during a duplicate determination Some hyperventilation must therefore be accepted during the $N_2$-wash-out procedure in a child

FRC and wash-out volumes were determined with satisfactory reproducibility errors of single determination were respectively 4 6 and 7

LCI was not biased by sex age or height However a significant decrease in LCI with increasing FRC was shown The mean value was 8 3 with a S D of 0 90 The error of a single determination was about 7 and the reproducibility was therefore satisfactory

Svanberg L *Scand J Cl Lab I est Suppl* 5 1957

Vivianne Ovelius *Quantitation of immunoglobulins in human serum from patients with mycoplasma infection*

In 17 patients—13 children aged 5 to 12 and 4 adults—with the diagnosis of primary atypical pneumonia the immunoglobulin levels in serum were determined by Oudin s single diffusion technique The IgM level was increased in all patients while the IgG level and the IgA level were normal The diagnosis was verified in all patients by x rays of the lungs mycoplasma pneumoniae cultivation mycoplasma pneumoniae complement fixation test cold agglutination test and strepto-coccus Mg agglutination test The IgM remained at a high level for at least 2 months after onset of the disease

Gunnar Meeuwisse *Stool chymotrypsin as a screening test of the exocrine function of the pancreas*

Chymotrypsin in random samples of faeces was measured according to Haverback in a pH stat (Radiometer Copenhagen) with N acetyl L tyrosin ethylester (British Drug House) a substrate In 117 tests carried out on 65 children—half of them younger than one year—chymotrypsin activity was always more than 75 μg chymotrypsin per gram faeces In stools from 14 children with non pancreatic diarrhea the mean chymotrypsin activity was about half of that in children without diarrhea In children with cystic fibrosis of the pancreas the stool always contained less than 75 μg chymotrypsin per gram usually only 10 μg or less The values obtained are in good agreement with those from the literature The activity towards the substrate was hardly affected by storage of the stool samples at room temperature for several days

Gunnar Engleson *Treatment of rheumatoid arthritis and nephrotic syndrome with immunosuppressive agents*

We have been very reluctant to use these potent immunosuppressive agents in children but about 10 cases of steroid resistant nephrotic syndrome rheumatoid arthritis LED and other collagen dis

placements in the electrolyte balance and radiography of the thorax did not reveal any infiltrates Twenty hours after the accident the patient could sit up in bed react to questions and eat During the subsequent days however she became lethargic had tremor and muscular spasms and attacks of *restlessness and terrified screaming* The symptoms suggested a profound cerebral and brainstem lesion The severely abnormal EEG gradually returned to normal and the mental and somatic states improved during training On discharge seven weeks after the accident psychological investigation showed the intelligence to be within normal limits the speach showed evidence of improvement and the fine movements were slightly uncertain One year after the accident the patient appeared to be perfectly normal

Two features are striking in reviewing this case history

1 The late deterioration, probably due to secondary traumatic cerebral oedema
2 *The final result compared with the condition* on admission A series of fortunate circumstances contributed to this No aspiration into the lungs treatment commenced at the scene of the accident cooling of the patient with resultant reduction of the oxygen requirement

E Nathan J Diderichsen & S Weihe *Pseudo hypoparathyroidism*

Pseudohypoparathyroidism is a rare condition Until 1964 a total of approximately 70 cases had been described The condition resembles idiopathic hypoparathyroidism but differs from this in that treatment *with parathyroid hormone* has no effect

A boy aged five years was described

Great mental improvement occurred during treatment with calciferol This has not been described previously

S Vestmark *Silver's syndrome*

Two cases of Silver's syndrome which were observed in the Pediatric Department Glostrup Hospital are reported

1 A girl born at term Birth weight 1370 g At nine months she was only 61 cm long and weighed 4380 g She had left sided hemihypertrophy and absence of all the toes on the left foot Chromosome investigation showed normal results Gonadotrophin excretion was normal

2 A boy born at term Birth weight 2250 g At five months he was 57 cm long and weighed 4090 g He had right sided hemihypertrophy and syndactyly on the hands and feet The fifth finger was short and crooked Gonadotrophin excretion was normal (These cases will be published elsewhere)

*Discussion*

*E Thamdrup* commented upon the somewhat confused picture of sexual development in these patients

S Vestmark *A case of myopathy for diagnosis*

A film was shown of a girl aged 10 years with a greatly incapacitating muscular condition

Muscle biopsy showed abnormal results but classification was difficult

The creatinine phosphokinase content in the serum was greatly increased

*Discussion*

*J Melchior* suggested secondary myopathy following arthrogryposis

*P Plum* Clinically the case resembles one of muscular dystrophy but the contracture of the hips confuses the picture The histology and biochemistry are as so often of little assistance

*E Wamberg* Patients of this type with severe contractures were seen previously in the Care of the Mentally Retarded prior to the introduction of energetic physiotherapy

*P Perregaard*

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cases were treated with these agents All the cases showed improvement although we obtained no permanent cures There were no dangerous side effects

N W Svenningsen *Some aspects of renal tubular function in premature infants during the first weeks of life*

A special type of metabolic acidosis ( late metabolic acidosis ) occurring in premature infants during the 2nd and 4th week of life is caused by a temporary disproportion between renal hydrogen ion excretion capacity and the load of non volatile acids This load in turn largely depends on the composition of the diet particulary the protein and mineral content Premature infants who are especially prone to develop this type of metabolic acidosis have all been found to have acid base value in the lower range of normal for premature infants during the first weeks of life By performing a single dose ammonium chloride load (54 mEq per sq m of body surface) it was found that premature infants with late metabolic acidosis (10 cases) had a smaller rise in renal hydrogen ion excretion capacity within the time limits of the test than premature infants (8 cases) The loading tests were performed at 2 to 3 weeks of age in all cases Average acid base values of premature infants therefore do not reflect the special susceptibility for development of metabolic acidosis in premature infants with an incompletely developed immature hydrogen ion excretion ability by the ammonium chloride loading test This must be taken into consideration when drawing up the feeding schedule for premature infants

## Meeting Febr 16–17 1968

Katherine Strangert *Comparison of the effects of using chloramphenicol and ampicillin in the treatment of pertussis*

At Roslagstulls Hospital Stockholm since 1962 half of the children treated for pertussis have been given chloramphenicol and the other half ampicillin in doses of 20–25 mg/kg four times a day for six days Infants under six months old were also given immunoglobulin as a measure against pertussis In the first group there were 72 infants and in the second group 76 of whom 17 and 21 respectively were newborns

Among those treated with chloramphenicol 6 of 48 patients had positive pertussis cultures even after therapy Corresponding figures for ampicillin were 15 and 47

Secondary infections with pneumococcus haemophilus influenzae beta streptococcus and staphylococcus aureus were encountered in three quarters of the children In about three quarters of the cases these organisms disappeared during treatment regardless of therapy

The incidence of coughing was noted daily This decreased when therapy was introduced in both groups but most markedly in the chloramphenicol group The difference however was not significant

As regards weight or decrease in the number of white blood cells no difference could be shown

The commonest side effect was diarrhoea with 10 and 15 cases respectively in the chloramphenicol and ampicillin groups Among the newborns the incidence of diarrhoea was equally high but of such severity in the ampicillin group that therapy in a number of cases had to be discontinued One newborn developed an exanthematous eruption during treatment with chloramphenicol Four children above the age of one year developed a mild skin eruption 1–7 days after initiation of therapy with ampicillin No other side effects were observed

Bengt Eriksson P Muller Brunotte & G Wallgren *Bacterial endocarditis*

Two boys 6 and 9 years old with previously healthy hearts had alpha streptococci with infection of the aortic valves Both had definite aortic insufficiency The younger boy died of metastatic brain embolism In the older boy the infection was arrested but he suffers from a pronounced valvular aortic insufficiency which required treatment with digitalis Autopsy revealed many bacteria in

the aortic valves notwithstanding five weeks of intensive intravenous therapy with penicillin doses amounting to 20 million I E per day

B Bjarke & Kerstin Thodenius *Late congenital neurosyphilis*

Neurosyphilis among pediatric patients is now a rarity This fact together with the varying symptomatology and course of the disease renders it easy to overlook the diagnosis This is illustrated by two patients in whom the diagnosis was made at the ages of 13 and 18 at Crown Princess Lovisa's Pediatric Hospital Both patients had previously been investigated and treated in hospital

*Case 1*

Born March 12 1954 *History* Patient No 2/2 *Family history* Mother Wasserman negative TPI positive

*Present illness* 1954 Slightly increased head circumference widening of lateral ventricles on encephalogram anaemia elevated sedimentation rate 1954–64 Progressive dementia and rigidity 1964 Severe headaches Rigid pupils not reacting to light Lumbar puncture—monocytes 162 protein 48 mg Progressive changes on encephalogram 1966 Stereotypic behaviour disorders of consciousness grand mal attacks Additional lumbar punctures unchanged Encephalogram (No 3) unchanged 1967 13 year old boy with severe mental retardation *Neurological findings* Absent patella and Achilles reflexes Maximally dilated fixed round pupils not reacting to light or accommodation Babinski positive bilaterally Left sided hemiplegia

*Case 2*

Born March 15 1949 *History* Patient No 2/5 *Family history* Unknown Mother Wasserman negative TPI positive *History* Gestation 33 weeks birthweight 600 grams Minor cleft palate Slightly retarded

1960 attended school for retarded children 1964 acute onset of confusion Pupils of differing size Possible positive Babinski on right side Stiff neck Lumbar puncture total number of cells 14 i.e. monocytes protein 84 mg/100 Transferred to infectious unit as possible meningitis 1965 placed in institution ... disturbed and forgetful Parkinsonism Status 1967 Eighteen year-old girl with severe mental retardation normally developed for her age Cachectic Mute No contact possible No definite reaction to noise Becomes hyperpnoeic when restless Slow athetotic movements Increased muscle tone Cogwheel phenomenon *Neurological findings* Hyperactive arm and leg reflexes equal Persistent patellar clonus Fixed round pupils unresponsive to light Babinski positive bilaterally

Approximately 10% of patients with untreated congenital syphilis subsequently developed neurosyphilis The first symptoms may appear as early as the age of 3–4 months in the form of meningitis Other symptoms include progressive dementia epilepsy pupillary anomalies hydrocephalus confusion ataxia etc

As the symptoms of neurosyphilis particularly in its congenital form vary and are non specific the old rule should be followed of excluding syphilis in all questionable neurological cases A Wasserman should be obtained in all pregnant women

It would seem advisable to determine whether children registered at the Central Board have had a Wasserman test

J Brohult G Andersson & G Sterner *Lactate formation and increased metabolic acidosis in two children following parenteral administration of fructose*

Parenteral infusions with a high fructose content are often used for patients requiring intravenous feeding The fructose becomes largely metabolised into lactate in the liver which results in metabolic acidosis The two cases reported below show that this acidosis is of practical clinical importance

*Case 1* A one year-old boy suffering from gastro-enteritis was admitted to the infectious diseases unit at Danderyd Hospital On admission he was dehydrated and had metabolic acidosis Parenteral administration of fructose was initiated corresponding to a dose of 3 g per kg body weight/hour which 7 hours later was reduced to 1 g per kg body weight/hour In spite of administration of large doses of bicarbonate the pH and standard bicarbonate further decreased during therapy and after 3 hours the patient lost consciousness After 5 hours the pH was 6 6 Not

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0 plasma as well as A or B blood corpuscles Antenatal prevention includes ABO grouping of mothers The prediction value of current immunization tests for the need of exchange transfusion is insufficient but a positive AB-gamma test according to Fischer implies a 33 % risk Postnatal prevention includes bilirubin analysis as well as serological examination as soon as early or unusually strong jaundice is observed

Th Ehrenpreis *Mortality in Hirschsprungs' disease in infancy*

Sixty six patients under 1 year of age have been treated consecutively from 1948 through 1966 for *Hirschsprung's disease* Two-thirds of the patients were admitted to hospital before the age of 21 days One patient died prior to definitive surgery and 3 after rectosigmoidectomy a mortality rate of 6 per cent During the last 8 years 37 patients were treated without mortality

An analysis of the relatively high mortality rates (20 to 43 per cent) in four recent similar series (Hofmann & Rehbein, 1966 Pellerin 1966 Shim & Swenson 1966 Fraser & Wilkinson 1967) suggests that the majority of deaths were due to environmental factors responsible for inadequate diagnosis or treatment prior to admission and/or to severe forms of enterocolitis Factors inherent in the disease and age were only of minor importance

The present series demonstrates that mortality rates are negligible under ideal conditions including early transfer of all infants with intestinal obstruction to specialized pediatric surgical centers and absence of serious problems from enterocolitis Ideal conditions are promoted by highly developed social welfare and medical care programs and by a relatively benign bacteriological environment

## Meeting March 23 1968

Hans Grahnen *Enamel hypoplasia in the primary set of teeth*

Following a summary review of etiological factors in enamel hypoplasia an account was given of an examination of children to mothers with diabetes in the 3–5 years agegroup plus a control group A total of 39 infants were delivered after an average pregnancy of 36 weeks and 44 were delivered after 38–40 weeks In the first group the incidence of enamel hypoplasia was 33 and in the second group 9 (the difference was significant at the 2 level)

In the control group (33 infants) the incidence was 6 There was a difference between the groups as regards duration of pregnancy which was terminated prematurely for women with diabetes The reason for the difference in the incidence of enamel hypoplasia between these groups may partly be that one of them was premature but the finding of prenatal hypoplasia showed that the mother's disease also played a role

As the infants in this study were premature the greater susceptibility of such infants to asphyxia was advanced as an explanation of the higher incidence of enamel hypoplasia A comparison was made between a control group of 56 infants and a group of 102 infants in the 2–3 year agegroup who had intrauterine asphyxia or asphyxia during the neo-natal period The incidence of enamel hypoplasia was higher in the asphyxia group than in the control group but the difference was significant only at the 5% level An attempt was made to classify the degree and duration of asphyxia but no significant relation could be shown between the incidence of enamel hypoplasia and the severity or duration of asphyxia—possibly because of the difficulty of evaluating these parameters

Gosta Samuelson & Stig Sjolin *Treatment of Wilson's disease with penicillamine*

A 12 year old girl was admitted to the Department of Paediatrics in Umeå at the age of 9 years because of severe cirrhosis of the liver with portal hypertension and ascites She had a low serum ceruloplasmin and low serum copper concentration a high urinary copper excretion and an in

withstanding anti shock treatment and external cardiac massage the patient died 24 hours later Autopsy revealed severe gastroenteritis and an undiagnosed brain tumour It is possible that the main cause of death was irreversible cell damage caused by acidosis

*Case 2* Two weeks later the same unit admitted a 2 year old girl suffering from stomatogingivitis with ulcerative lesions On admission she was de hydrated and had metabolic acidosis Parenteral administration of fructose was initiated cor responding to a dose of $1^1/_2$ g per kg body weight/hour In spite of administration of bi carbonate pH and standard bicarbonate further decreased during treatment and the girl gradually lost consciousness When intravenous fructose was discontinued 5 hours later her condition steadily improved during shock therapy Her survival can probably be attributed to the timely termination of parenteral fructose

In view of these two cases and the findings of Bergstrom & Hultman we recommend a re appraisal of the suitability of fructose for pa renteral use and advise against its use at least for patients likely to develop acidosis and tissue hy poxia

G Sterner & Gunnel Biberfeld *Mycoplasma pneu moniae infections Family Studies*

The material currently includes 12 families to talling 44 persons 18 of whom were under 15 years of age The index case was a family member admitted to the infectious diseases unit a Dan deryd Hospital who proved to have a mycoplasma pneumoniae (MP) infection

Mycoplasma pneumoniae was isolated from 78% (33/42) of the family members examined Most of them excreted mycoplasma over a pe riod of many weeks in spite of treatment with erythromycin and tetracyclin Excretion of MP was observed in one child $\geq 8$ weeks

There was good correlation (90%) between significant (> fourfold) antibody increase against MP (22 cases) and isolation of MP in these cases (20 cases)

Eighty six per cent (38/44) of the family members at that time or shortly before had an MP infection if the growth of MP and/or a significant titer increase in antibodies against MP or a high ($\geq 64$) unchanged titre is taken as th criterion for a current or a recent MP infection

Of the 44 members of this group only 5 wer healthy—4 of these having no demonstrable ex cretion of MP and 3 having low unchanged titres against MP The remaining 39 were sick with various symptoms and clinical findings from the respiratory tract In over half of the patients (25) pulmonary infiltrations of pneumonic type were verified by roentgenological examination The in cubation period was 14–23 days

Birgitta Jalling Birger Broman & Per Zetterqvist *AB0 immunization as a cause of bilirubin ence phalopathy*

Eighteen cases of bilirubin encephalopathy oc curred among 2778 newborns admitted to the pae diatric clinic at Karolinska Sjukhuset 1958–1967 because of haemolytic disease or hyperbilirubi naemia Four were ascribed to D immunization and four to prematurity without blood group in compatibility In the remaining 11 cases AB0 incompatibility was present the mothers belong ing to blood group 0 and the infants to group A (9 cases) or B (2 cases) without associated RH incompatibility In all cases symptoms of ence phalopathy were manifest on admission and in all except one death or severe permanent brain damage Two of the infants had had exchange transfusions prior to admission Group 0 blood had been given in one case of A immunization and group B blood in one case of B immunization In both cases the serum bilirubin had continued to increase after treatment It cannot be stated whether this was due mainly to the choice of donor blood or whether it might be related to haemolysis following over heating of the bottles

*Our experience brings up the problems involved* in prevention of brain damage related to AB0 incompatibility Possibilities for frequent and ade quate bilirubin analysis and for repeated exchange transfusions at short intervals must be available in every case of threatening bilirubin encephalo pathy Centralization to hospitals serving a large population is necessary to keep a satisfactory standard In cases of AB0 incompatibility group 0 blood corpuscles suspended in plasma correspond ing to the infant s blood group (or AB plasma) should be given to avoid administration of group

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creased copper deposition in the liver A Kayser Fleischer ring was found in the cornea but there were no signs of lenticular degeneration Treatment with penicillamine initially 1 g per day and later 0 75 g resulted in rapid improvement After 1 year s therapy the Kayser Fleischer ring had disappeared After 2 $^1/_2$ years the girl was symptom free All liver function tests were normal a liver biopsy showed very slight signs of cirrhosis and copper could no longer be demonstrated

Karl Martin Lundmark *Proliferation of bone marrow cells during initial remission stage of acute leukemia in children treated with corticosteroids*

Eight children with acute blast leukemia were followed with repeated blood and bone marrow examinations during the initial remission stage induced by prednisone alone The bone marrow samples were incubated with tritiated thymidine in vitro and the proliferative activity of leukemic and normal bone marrow cells was estimated by autoradiographic technique All children had a complete remission The results indicate that leukemic blast cells have a very low proliferative activity compared to normal stem cells and that erythropoietic and granulopoietic cells proliferate normally compared to corresponding cells in healthy children (cf *Acta Paediat Scand* Suppl *162* 1966) Neither leukemic nor normal bone marrow cells seem to change their proliferation pattern during the remission stage The corticosteroids probably increase the proportion of leukemic blast cells that differentiate into normal cell lines The main defect of the leukemic blast cells is apparently their inability to differentiate into normal cell lines

Orvar Finnstrom *Different methods for estimating the degree of maturity of newborn infants*

How to measure the degree of maturity of newborn infants was discussed Special attention was focused on the neurological examination used by Saint Anne Dargassies In study of a sample of infants at the Department of Paediatrics University of Umea several methods for evaluating maturity were employed and the material subseq processed by computers Certain antropometric data different external characteristics neurological maturity signs results from roentgenological examination of epiphysial centers and finally the results from measuring motor nerve conduction velocities were registered

Gunther Koch & Hans Wendel *The effect of pethidine on the postnatal adjustment of respiration and acid base balance* (To be published in *Acta Obst Gynec Scand 47* 1968)

Sigfrid Blom & Orvar Finnstrom *Motor nerve conduction velocities in newborn infants of various gestational ages* (To be published in *Acta Paediat Scand 57* 1968)

Orvar Finnstrom & Hans Wendel *Experiences of active treatment of respiratory difficulties in newborn infants* (To be published later)

Andreas Killander Karl Martin Lundmark & Stig Sjolin *Idiopathic aplastic anaemia in childhood* (To be published in *Acta Paediat Scand*)

*R Lagercrantz*

# NEW BOOKS RECEIVED

J Ströder & W Henle (eds) *Probleme der Verhütung von Virenkrankheiten* 212 pp Springer Verlag Berlin Heidelberg & New York 1967

M P König *Die kongenitale Hypothyreose und der endemische Kretinismus* 175 pp (with 20 figs) Springer Verlag Berlin Heidelberg & New York 1968 DM 58 US $14 50

O Gsell *Antibiotica et chemotherapia* vol 14 280 pp Karger Basel & New York 1968 sFr/DM 69

E Rossi (ed) *Orthopaedische Fragen in der Paediatrie* (Pädiatrische Fortbildungskurse für die Praxis) vols 5–6 198 pp (with 111 figs) Karger Basel & New York 1968 sFr DM 39

P Mozziconacci J Boisse A Lemonnier & C Charpentier *Les maladies métaboliques des acides aminés avec arriération mentale* 393 pp L'Expansion Scientifique Paris 1968 51 Fr

O Tonz *The congenital methemoglobinemias* (Bibliotheca Haematologica No 28) 146 pp (with 28 figs) Karger Basel & New York 1968 sFr DM 39

H Opitz & F Schmid (eds) *Handbuch der Kinderheilkunde* Vol 9 *Pädiatrische Grenzgebiete Augen Ohren Zähne Haut* (ed H Mai) 968 pp Springer Verlag Berlin Heidelberg & New York 1968 DM 383

F Linneweh (ed) *Fortschritte der Pädologie* vol 2 246 pp (with 72 figs) Springer Verlag Berlin Heidelberg & New York 1968 DM 98

A J Moss & F H Adams (eds) *Heart disease in infants children and adolescents* 1140 pp Williams & Wilkins Baltimore 1968 US $48 50

R Torpin *Fetal malformations Caused by amnion rupture during gestation* 165 pp Thomas Springfield Ill 1968 US $11 50

H B Marsden & J K Steward (eds) *Recent results in cancer research* Vol 13 *Tumours in children* 347 pp (with 258 figs) Springer Verlag Berlin Heidelberg & New York 1968 US $18

P Kildeberg *Clinical acid base physiology* 228 pp Munksgaard Copenhagen 1969 Dan kr 75

R Mackeith & M Bax (eds) *Studies in infancy* (Clinics in Developmental Medicine No 27) 109 pp William Heinemann Medical Books London 1968 25s

N Neimann J Montaut & J Sapelier *Épanchements sous-durals chroniques du nourrisson* 208 pp (with 49 figs) L'Expansion Scientifique Paris 1968 30 Fr

C Sauter (ed) *Kinderpsychiatrie in der Praxis* (Pädiatrische Fortbildungskurse für die Praxis) vol 9 114 pp Karger Basel & New York 1969 sFr/DM 24

V Dubowitz *Developing and diseased muscle* (S.I.M.P. Research Monograph No 2) 107 pp William Heinemann Medical Books London 1968 30s

P Geertinger *Sudden death in infancy* 107 pp Thomas Springfield Ill 1968 US $6 75

D J Beintema *A neurological study of newborn infants* (Clinics in Developmental Medicine No 28) 178 pp William Heinemann Medical Books London 1968 37s

H F R Prechtl & D J Beintema *Die neurologische Untersuchung des reifen Neugeborenen* 81 pp Thieme Stuttgart 1968 DM 14 80

G A von Harnack (ed) *Kinderheilkunde* 451 pp Springer Verlag Berlin Heidelberg & New York 1968 DM 38

R Richterich *Klinische Chemie Theorie und Praxis* 542 pp Karger Basel & New York 1968 US $18 sFr 75

M D Ebel & E Willich *Die Röntgenuntersuchung im Kindesalter Technik und Indikation* 289 pp (with 67 figs) Springer Verlag Berlin Heidelberg & New York 1968 DM 118

G Joppich & F J Schulte *Neurologie des Neugeborenen* 589 pp (with 153 figs) Springer Verlag Berlin Heidelberg & New York 1968 DM 138

J Wawersik *Ventilation und Atemmechanik bei Säuglingen und Kleinkindern unter Narkosebedingungen* (*Anaesthesiology and Resuscitation* vol 24) 151 pp (+84 figs) Springer Verlag Berlin Heidelberg and New York 1967 DM 32 US $8

P H Sandifer *Neurology in Orthopaedics* 63 pp Butterworth & Co Ltd London 1967 16s

E Rossi & E Stoll (eds) *Biochemistry of Glycoproteins and Related Substances* (*Cystic Fibrosis* part II) Proc 4th Int Conf on Cystic Fibrosis of the Pancreas (Mucoviscidosis) 329 pp (+75 figs) S Karger AG Basel and New York 1968 SFr /DM 75 150s

S Z Levine (ed) *Advances in Pediatrics* vol XV 288 pp Year Book Medical Publ Inc Chicago 1968 U S $12 50

J G Millichap *Febrile Convulsions* 222 pp Collier Macmillan Ltd London and New York 1968 75s

P A Voûte *Neuroblastoom Ganglioneuroom Phaeochromocytoom* 137 pp A Oosthoek's Uitgeversmaatschappij N V Utrecht 1968 Dfl 25

J K Lattimer P F Bischoff & W Gregoir (eds) *Urinary Diversion in Children* (Pediatric Urology Meeting July 15–17 1967 Starnberg (Munich)) Sep *Urologia Internationalis* 23 Nos 1–2 1968 198 pp (+130 figs) S Karger AG Basel and New York 1968 sFr /DM 29 58s

# BOOK REVIEWS

P H Sandifer *Neurology in Orthopaedics* 63 pp Butterworth & Co Ltd London 1967 16s

In 63 pages Dr Sandifer has summarized his vast experience of neurological disorders presenting orthopaedic problems The book reviews briefly but exactly most of the diseases and injuries of the neuromuscular system which may cause movement disorders It is divided in three parts each dealing with the problems arising in a limited age group infancy childhood and adulthood Two thirds of the book is concerned with the diseases affecting infants and children thus reflecting Dr Sandifer s great interest in these age groups The book must be an excellent source of information for orthopaedic surgeons for whom it is obviously written as it gives in a condensed and clear way the medical neurological background to many of the problems the orthopaedic surgeon may be liable to treat as a question of angles and levers It is perhaps a drawback that Dr Sandifer does not always state in which situations the patient may benefit from operations and when such procedures should be avoided as they or the bed rest following them may cause deterioration Physicians interested in how to establish a diagnosis of the discussed disorders may miss exact directions on this point many readers may also deplore the complete lack of illustrations However it has obviously not been the author s intention to write a complete text book of movement disorders The book covers in a nice condensed form the area indicated by the title and can within this area by highly recommended

*Ingrid Gamstorp*

A Rusescu I Balaban & V Popescu *Diagnostic Clinic şi Radiologic in Pediatrie* 1457 pp Three vols Editora Didactica şi Pedagogica Bucharest 1967

This book is the result of a study of the case material assembled over a 25 year period in the 1st Pediatric Clinic of Bucharest It is essentially an atlas in which the radiographic illustrations throughout the three volumes are presented along with clinical comments and extensive case histories The text is in Rumanian and translated into French and English The case histories are richly supplemented by electrocardiograms and illustrations of hematologic and pathologic specimens several of them in colour There are more than 1300 reproductions of radiograms of which many are composite illustrations and aimed to cover the findings in the respiratory cardiovascular renal alimentary and osseous systems They certainly contain a wealth of information and the well arranged case histories help considerably the reader in the interpretation of the radiographs However it is to be regretted that the quality of the reproductions too often is poor by usual standards and does not allow the details to stand out with desirable clarity Also differential radiologic diagnosis is not given The reviewer who is fully aware of the difficulty to create a proper balance of space allotted to the different topics in a publication of this kind is nevertheless surprised to find that there is no particular section dealing with the lungs in newborns fractures of the skeleton and abdominal emergencies In addition no reference is made to the pathology of the lower urinary tract There is no bibliography and no index Unfortunately in view of the above mentioned deficiences it is hard to recommend the monograph unrestrictively despite its many merits

*Ulf Rudhe*

J Wawersik *Ventilation und Atemmechanik bei Säuglingen und Kleinkindern unter Narkosebedingungen* 151 pp illus

Springer Verlag Berlin Heidelberg and New York 1967 DM 32

A rather extensive review of the theoretical considerations of mechanics of breathing and respiratory work forms the introduction to some of the main problems in anesthesia for infants and children

Under the heading of Ventilation the relationship between such parameters as frequency tidal volume and flow rates is presented based on results from studies of 105 children under anesthesia for minor surgical procedures The age of the children ranged from 4 weeks to 9 years Among a considerable mass of useful data often displayed in clear graphs it is stated that the tolerance limits for additional dead space from face masks and valves are very low in infants and are often exceeded by the conventional anesthesia equipment

The relationship between respiratory work and resistance to breathing caused by face masks and endotracheal tubes using spontaneous and controlled respiration is considered under the heading of Mechanics of Respiration

The author touches on some of the principles of respirator treatment by considering the relationship between time of inspiration and expiration and to some extent the matter of frequency The conclusions seem somewhat hasty however since the performance of respirators depends on many factors not mentioned among them the flow pattern generated by the respirator

By his studies the author has supplied valuable data hitherto lacking thus confirming the empirical knowledge that considerable care must be exercised when chosing anesthetic equipment for and giving anesthesia to small children

*Hans Feychting*

H F Brissaud R Laplane J Lafourcade P Mozziconacci A Rossier P Royer & S Thieffry (eds) *Journées Parisiennes de Pédiatrie 1967* 416 pp illus Éditions Médicales Flammarion Paris 1967 Fr 80

Enfants-Malades in Paris The lectures presented are now collected in this book and are very quickly published and given a typographically attractive design

The book is divided in three parts general paediatrics the problems of the first year of life and recent knowledge about immunoglobulins Some details of the subjects which were presented at the meeting will be given

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The relationship between respiratory work and re sistance to breathing caused by face masks and endo tracheal tubes using spontaneous and controlled re spiration is considered under the heading of Mechan ics of Respiration

The author touches on some of the principles of respirator treatment by considering the relationship between time of inspiration and expiration and to some extent the matter of frequency The conclusions seem somewhat hasty however since the performance of respirators depends on many factors not men tioned among them the flow pattern generated by the respirator

By his studies the author has supplied valuable data hitherto lacking thus confirming the empirical knowledge that considerable care must be exercised when chosing anesthetic equipment for and giving anesthesia to small children

*Hans Feychting*

## ANNOUNCEMENT

The Second National Congress of Pediatrics will be held in Bucharest Romania May 8-10 1969 Information concerning the congress can be obtained through The Secretariat of the Second National Congress of Pediatrics Union of Societies of Medical Sciences Str Progresului no 8-10 Bucuresti 45 Romània

## EUROPEAN SOCIETY FOR PEDIATRIC RESEARCH

The European Society for Pediatric Research was founded in Vienna on August 26 1968 by the unanimous vote of the members of the European Club for Pediatric Research The main objective of the new Society will be to promote pediatric research in Europe by the interchange of ideas and information between investigators in the field of pediatrics

The first scientific meeting of the new Society will be held at Bern Interlaken (Switzerland) on Sep ember 15-17 1969

The official languages will be English French and German

Active membership will be open to any person who has made original contributions in pediatric res arch The age limit for active members is 45 years (except for the first year of the Society when persons up to 50 years can apply for membership)

Professor Ettore Rossi (Bern) was elected president of the Society

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Furth r information about the Society may be obtained from Dr Fabio Sereni Clinica Pediatrica del l'Università, via Commenda 9 20122 Milan Italy

lack behind the research frontier. Since the book was published the role of local immunity conveyed by immunoglobulin A and the possibilities to develop it by local administration of respiratory virus vaccine have been investigated.

*Rutger Lagercrantz*

Poul Kildeberg: *Clinical acid base physiology.* 228 pp. Munksgaard, Copenhagen 1968. Dan. kr. 75.

Denmark has an old tradition of being the leading country within acid base research, as manifested by names such as Hasselbalch, Warburg, Brønsted, Astrup and Siggaard Andersen. Kildeberg joins his compatriots by writing a monography mainly dealing with acid base problems in pediatrics. He started out in cooperation with Astrup and Siggaard Andersen in Copenhagen and is now in the same department as Winters and Engel at Columbia University in New York. It follows that the book is full of acid base learning. It is no easy reading even for someone who has already taken his first steps within this field.

Kildeberg has an excellent point in not only doing the traditional blood gas an acid base analyses but of measuring at the same time the elimination of total acids and $NH_4$ in the urine. The latter measurements are done with a Radiometer Titrigraph. It is by no means only his chapter on kidney diseases which profits from this approach. It is also valuable for instance when he discusses the relationship between metabolic acidosis in the neonatal period and the protein feeding.

The chapter on the acid base regulation of the kidney both under physiological and pathological conditions is extensive and most informative. Another main area covers the newborn period. Acid base measurements on 109 premature infants during the 2nd and 3rd week of life show such a range that it is virtually impossible to speak of normal values even in otherwise normal infants. However during the first weeks the values are better clustered and normal values may therefore be attempted for this period. This type of studies is important in showing all those who now make routine analyses of acid base balance on the premature that there are no rigid rules for normal values. It is therefore more important to follow changes than to draw any specific conclusions from the individual level.

Kildeberg has a large material of infants with pyloric stenosis. They are treated preoperatively with ammonium chloride which normalizes their metabolic alkalosis. He shows that in the postoperative period the nonrespiratory alkalosis invariably returns for a few days.

Particularly interesting data are presented regarding the degree of respiratory compensation by metabolic alkalosis as there are people who maintain that no such compensation occurs.

The main value of this book will be as a source of reference on perinatal wards. He who has the opportunity to understand the text will learn much about acid base problems.

*Gosta Rooth*

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*Acta Pædiat Scand* 58 109–115 1969

# ALBUMIN METABOLISM AND GASTROINTESTINAL PROTEIN LOSS IN CHILDREN WITH NEPHROTIC SYNDROME

MINNA YSSING HERLUF JENSEN and STIG JARNUM

*From the Paediatric Department and Medical Department P Division of Gastroenterology Rigshospitalet Copenhagen Denmark*

Isotope studies of plasma protein metabolism in the nephrotic syndrome in both children (11 31) and adults (1 2 6 8 9 10 15 18 20 22) have revealed an increased fractional endogenous catabolism of albumin transferrin and IgG which apparently is unassociated with urinary protein loss In some patients in particular children an abnormal gastrointestinal protein loss has been claimed to account for the endogenous hypercatabolism of plasma proteins (7 21 23 28 29 30) In other cases protein loss by the intestinal route could not be accepted as a significant factor (19)

Quantitation of gastrointestinal protein loss has been difficult to assess due to lack of suitable methods for this purpose Especially in children urinary contamination of the stools represents a source of error when one employs isotopes which are also excreted by the kidneys With $^{59}$Fe labelled iron dextran a new test substance has been introduced for detection and within limits quantitation of gastrointestinal protein loss It offers several advantages $^{59}$Fe is present in a stable bond and the isotope is not excreted in the urine to any significant degree Accordingly false positive tests resulting from urinary contamination of faeces are eliminated For obvious reasons this makes $^{59}$Fe iron dextran practical in paediatric studies The present investigation was undertaken to throw further light over the quantitative role of gastrointestinal protein loss in children with nephrotic syndrome

## METHODS

Serum albumin was measured immunochemically (27) alfa$_2$ globulin by paper electrophoresis (26) Proteinuria (g per 24 hours) was determined by Kjeldahl's method in 3 to 8 days and the average value used for assessment of renal protein loss

In all patients simultaneous albumin and IgG turnover studies were performed together with a $^{59}$Fe labelled iron dextran test The results of IgG turnover studies are published elsewhere

*$^{59}$Fe labelled iron dextran test* Being a non protein macromolecule with an average molecular weight of 180 000 $^{59}$Fe iron dextran offers an indirect method of estimating gastrointestinal protein loss Details of the method are reported elsewhere (5 16)

An intravenous injection of a known dose of $^{59}$Fe iron dextran was given The faecal output of the isotope was measured over a 4 day period The result was expressed as the *faecal clearance* of $^{59}$Fe (i e daily faecal output of $^{59}$Fe as percentage of the mean content of the isotope in the plasma within the same 24 hours) or as the percentage of the injected dose excreted in the stools during a 4 day collection period In normal adult subjects the faecal isotope clearance amounts to 0 to 0 8 per cent of the intravascular pool per day and less than 1 per cent of the injected dose is excreted in the stools during a 4 day period

*$^{131}$I-albumin turnover* Albumin turnover was studied with $^{131}$I labelled albumin Human albumin was obtained from Behringwerke Marburg Lahn Germany ("Albumin trocken rein") It was labelled with $^{131}$I with monoiodine chloride as oxydizing agent Physicochemical and metabolic characteristics of the preparation are given in details elsewhere (33)

A known dose of $^{131}$I albumin was injected intravenously Blood samples for plasma counting were withdrawn into heparinized tubes 10 minutes after

This work was supported by grants from Statens Videnskabsfond P Carl Petersens Fond and Kong Christian den X's Fond

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An intravenous injection of a known dose of $^{59}$Fe iron dextran was given The faecal output of the isotope was measured *over a 4 day period* The result was expressed as the faecal clearance of $^{59}$*Fe* (*i.e.* daily faecal output of $^{59}$Fe as percentage of the mean content of the isotope in the plasma within the same 24 hours) or as the percentage of the injected dose excreted in the stools during a 4 day collection period In normal adult subjects the faecal isotope clearance amounts to 0 to 0 8 per cent of the intravascular pool per day *and less than 1* per cent of the injected dose is excreted in the stools during a 4 day period

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A known dose of $^{131}$I albumin was injected intravenously Blood samples for plasma counting were withdrawn into heparinized tubes 10 minutes after

Table 1 *Laboratory data in 7 children with nephrotic syndrome*

| Age (yrs) | Sex | Hb (g/100 ml) | ESR (micro) (mm) | Serum cholesterol (mg/100 ml) | Serum creatinine (mg/100 ml) | Proteinuria (Kjeldahl) (g/day) |
|---|---|---|---|---|---|---|
| 2 1/12 | F | 12 0 | 2 | 542 | 0 6 | 3 30 |
| 2 5/12 | M | 14 0 | 1 | 582 | 0 9 | 1 05 |
| 3 8/12 | F | 11 1 | 22 | 644 | 0 5 | 1 01 |
| 3 7/12 | F | 13 8 | 20 | 506 | 0 6 | 1-2 * |
| 6 10/12 | M | 13 5 | 28 | 570 | 0 4 | 1 02 |
| 9 /12 | M | 12 5 | 69 | 535 | 0 7 | 1 62 |
| 11 9/12 | M | 14 0 | 5 | 644 | 0 6 | 7 90 |

Determined by the method of Esbach

the injection and at daily intervals over a period of 1–2 weeks Urine was collected in 24 hours specimens in the same period Stools were collected and homogenized in 24 hour specimens in a 4 day period following the injection

The radioactivity of plasma and urine was measured in 1–3 ml aliquots and faecal activity in weighed duplicates Proteinbound radioactive iodine in urine was determined after precipitation with 10 per cent trichloracetic acid Errors from the precipitation procedure was about 5 per cent (coefficient of variation) The radioactivity was measured in a three channel spectrometer (Autogammaspectrometer Packard) and compared to that of a standard solution containing a known fraction of the injected dose Each channel was set to measure the peak energy of either $^{59}$Fe $^{131}$I or $^{125}$I (in $^{125}$I IgG) The samples were counted for at least 10 minutes In the majority of samples the counting error (coefficient of variation) was less than 3 per cent Samples with a net count of less than 20 per cent of the background were considered to contain no significant amount of isotope Total protein concentration (biuret) was determined in all plasma samples in order to obtain an approximated specific activity (counts per minute per mg protein) As a control of the collection procedures the amount of $^{59}$Fe and $^{125}$I excreted was estimated by daily whole body counting (4)

Steady state condition was checked by daily weighing of the patients and determination of haemoglobin serum protein and albumin (paper electrophoresis) twice a week In order to prevent thyroid uptake of radioactive iodide released during catabolism of the labelled proteins the patients were given potassium iodide 3 to 5 mg twice a day (24)

About 0 04 µCi $^{59}$Fe 1 00 µCi $^{131}$I and 0 5 µCi $^{125}$I were injected per kg Assuming that the mean life time of the proteins was reduced to one third to one half of the normal value of 20–30 days the total body radiation dose amounted to about 120 mrem In normal children the total body radiation dose would have been about 320 mrem

*Analysis of data*

Plasma volume (*PV*) was calculated by division of the injected amount of $^{131}$I by the activity of $^{131}$I in 1 ml plasma 10 minutes after the injection The radioactivity of the plasma sample was corrected with a factor 1 02 to allow for initial extravascular deposition of the labelled protein (12 32)

Intravascular mass (*IVM*) of the protein equals *PV* × serum concentration

Distribution ratio (*D*) is the percentage of total albumin located intravascularly *D* was calculated after Nosslin s method (cited in ref 3)

Total mass (*TM*) equals (*IVM* × 100 g)/*D*

Fractional disappearance rate (*FDR*) is the percentage of *IVM* disappearing from the plasma per day by way of catabolism and external loss It was obtained by mathematical analysis of the plasma activity curve after Nosslin s method (3)

Fractional catabolic rate (*FCR*) is the percentage of *IVM* catabolized per day *FCR* equals [*FDR* × (100 − *PBI* )]/100 day$^{-1}$ where *PBI* indicates protein bound $^{131}$I as percentage of total urinary $^{131}$I

Mean life time (*MLT*) represents the average life time of a protein molecule in the organism Being equal to 1/(*FDR* × *D*) days *MLT* is negatively correlated to *FDR* and *D* (3)

Synthetic rate (*S*) Assuming steady state conditions the synthesis of albumin must equal the sum of catabolism and renal loss Hence *S* = (*IVM* × *FDR*)/100 g per day When *S* is given as mg per kg per day the oedema free weight was used i e weight before disease or following excretion of oedema

## CASE MATERIAL

Seven children with untreated nephrotic syndrome 4 boys and 3 girls between 2 and 11 years of age were studied According to clinical and laboratory criteria they all represented an active acute phase of nephrotic syndrome characterized by oedema dysproteinaemia massive proteinuria and hyperlipaemia Table 1 shows laboratory data including haemoglobin erythrocyte sedimentation rate serum cholesterol serum creatinine and proteinuria Table 2 presents serum concentrations of total protein albumin and alfa 2 globulin All children had severe hypoalbuminaemia and a greatly elevated alfa 2 globulin

Serum creatinine blood pressure urine sediment and intravenous pyelograms were normal with the excep

Table 2 *Serum concentration of total protein albumin and alfa 2 globulin in 7 children with nephrotic syndrome*

| Age (yrs) | Sex | Serum protein (g/100 ml) | Serum albumin (g/100 ml) | Serum alfa 2 globulin (g/100 ml) |
|---|---|---|---|---|
| 2 / | F | 4 33 | 0 35 | 2.21 |
| 2 /₁₂ | M | 2 87 | 0 44 | 1 43 |
| 3 / | F | 4 42 | 0 84 | 1 64 |
| 3 /₁₂ | F | 4 15 | 1 12 | 1 33 |
| 6 / | M | 4 00 | 0 93 | 1 72 |
| 9 / | M | 3 70 | 0 99 | 1 09 |
| 11 / | M | 3 72 | 0 77 | 1 52 |
| Normal values | | 6 60–8 20 | 3 55–4 99 | 0 31–0 59 |

tion that in one patient a duplication of pelvis and ureter was present on the right side In 2 patients who subsequently failed to respond to steroid treatment a renal biopsy was performed (boy 9 /₁₂ years and girl 2 / years) which in both showed glomerular changes with *proliferation of the capsular epithelium* and some degree of hyalinization which conformed to a picture of subacute glomerulonephritis

In the remaining 5 patients a diagnosis of genuine lipoid nephrosis was based on case history and clinical course of the disease including an optimal response to steroid treatment

## RESULTS

### *⁵⁹Fe labelled iron dextran test*

The results of ⁵⁹Fe iron dextran tests are shown in Table 3 Faecal output of ⁵⁹Fe expressed as percentage of the dose injected varied from 0 to 1 6 (normal range 0–1 0) In 4 patients faecal ⁵⁹Fe excretion was normal in the remaining 3 it was from 1 1 to 1 6 per cent of the injected dose

Faecal ⁵⁹Fe clearance varied from 0 to 2 7 per cent of the intravascular pool per day (normal range 0–0 8) In 3 children it was normal and in 4 slightly elevated from 1 6 to 2 7 per cent per day Urinary ⁵⁹Fe excretion was not detectable in 2 patients In the remaining 5 it varied from 0 4 to 2 3 per cent of the injected dose during a 4 day collection period following the injection

### *Albumin turnover*

The results of albumin turnover studies are presented in Table 4 The fractional disappearance rate (*FDR*) was elevated in all patients It ranged from 46 to 96 per cent per day

The fractional catabolic rate (*FCR*) varied from 21 to 61 per cent per day (normal range for this age group 6 to 12 per cent per day (25)) Thus *FCR* was markedly increased in every case

Distribution ratio (*D*) varied from 43 to 68 per cent (normal range 41–54 (25)) In 3 pa

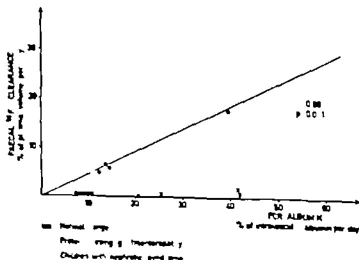



*Fig 1* Correlation between fractional catabolic rate (*FCR*) of ¹³¹I labelled albumin and faecal ⁵⁹Fe-clearance in 70 cases of protein losing gastroenteropathy and 7 children with nephrotic syndrome In the nephrotic patients the endogenous fractional catabolic rate is listed.

Table 3 *[59]Fe labelled iron dextran test in 7 children with nephrotic syndrome*

| Age (yrs) | Sex | Faecal [59]Fe output in 4 days of injected dose | Faecal [59]Fe clearance of plasma vol /day |
|---|---|---|---|
| 2 1/12 | F | 0 5 | 0 8 |
| 2 6/12 | M | 1 1 | 1 7 |
| 3 3/12 | F | 0 9 | 1 6 |
| 3 7/12 | F | 1 6 | 2 7 |
| 6 10/12 | M | 1 3 | 1 6 |
| 9 /12 | M | 0 | 0 |
| 11 6/12 | M | 0 5 | 0 5 |
| Normal values | | < 1 0 | < 0 8 |

tients a normal *D* was found In the remaining 4 it was elevated

Mean life time (*MLT*) varied from 2 to 4 days (normal range 19–33 (25)) It was greatly reduced in all children

Synthetic rate (*S*) amounted to 147–359 mg per kg per day (normal range 120–200 (25)) *S* was normal in 3 cases and elevated in the remaining 4

## DISCUSSION

It is generally held that the reduction of serum albumin in the nephrotic syndrome is primarily due to urinary albumin loss However studies by Gitlin *et al* (11) showed that an associated increase of endogenous catabolism of serum albumin and several other plasma proteins in nephrotic children may contribute to the hypo proteinaemia Their results have been confirmed in more recent studies of plasma protein meta bolism in adult nephrotic patients (1 2 6 8 9 10 15 17, 18 20 22) and in Perheentupa s study from 1967 in children with congenital nephrotic syndrome (31) and in the present study of childhood nephrosis We found a very rapid turnover of serum albumin which could not be accounted for by urinary albumin loss The endogenous fractional catabolic rate was greatly elevated in some patients representing values more than 5 times the normal value Though similar findings have been made in adult nephrotic patients the elevation of the fractional catabolic rate is less pronounced in adults At present we have no explanation of this quantitative difference between children and adult patients

Distribution ratio mean life time total mass and intravascular mass as well as the synthetic rate of albumin in the present series agree well with results obtained in adult nephrotic patients (6 18 20) The high distribution ratio means that an abnormally high fraction of the total albumin mass is located intravascularly It agrees well with the fact that the albumin con

Table 4 *Albumin turnover data in 7 children with nephrotic syndrome studied with [131]I labelled albumin*

*FDR* – Fractional disappearance rate per cent of intravascular mass disappearing per day from the plasma by way of catabolism and external loss *FCR* fractional catabolic rate per cent of intravascular mass catabolized per day *D* – distribution ratio per cent of total mass located intravascularly *MLT* mean life time average life time of an al bumin molecule in the organism *S* – Synthetic rate

| Age (yrs) | Sex | Serum albumin (g/100 ml) | *FDR* | *FCR* | *D* | *MLT* Days | *S* g/day | *S* (mg/kg/day) |
|---|---|---|---|---|---|---|---|---|
| 2 1/12 | F | 0 35 | 96 | 42 | 64 | 1 6 | 2 09 | 147 |
| 2 6/12 | M | 0 44 | 86 | 61 | 43 | 2 7 | 2 21 | 177 |
| 3 3/12 | F | 0 84 | 67 | 56 | 51 | 2 9 | 3 66 | 240 |
| 3 7/12 | F | 1 12 | 69 | 44 | 68 | 2 1 | 5 39 | 359 |
| 6 1/12 | M | 0 93 | 57 | 42 | 63 | 2 8 | 6 42 | 238 |
| 9 /12 | M | 0 99 | 53 | 21 | 66 | 2 9 | 7 93 | 283 |
| 11 /12 | M | 0 77 | 46 | 26 | 51 | 4 3 | 6 37 | 152 |
| Normal range | | 3 55–4 99 | | 6 3–11 6 | 41–54 | | | 120–200[a] |

[a] From Kraulnikoff Andersen & Rossing (25)

centration in ascitic and oedema fluid is extremely low in nephrosis. It may reflect a decreased capillary permeability or more probably an increased lymphatic flow.

The problem remains at which site and by which mechanism the protein hypercatabolism takes place. Several studies have been published on gastrointestinal protein loss in nephrotic patients in particular children. In some of these investigations an abnormal gastrointestinal protein loss was detected and claimed to account for a significant part of the hypercatabolism (7, 21, 22, 23, 28, 29, 30). Using [131]I polyvinylpyrrolidone Bennhold & Ott in 1962, Nusslé *et al* in 1962, Kluthe *et al* in 1963 and Marchal in 1965 demonstrated an increased faecal excretion of [131]I after intravenous injection of the labelled compound in patients with nephrotic syndrome. By means of radioiodinated albumin and oral administration of an anion exchange resin Jimenez Diaz *et al* in 1963 and Nusslé & Royer in 1964 found an increased faecal excretion of the label in nephrotic patients. They concluded that abnormal gastrointestinal protein loss took place. In contrast to these results are findings by Jensen *et al* in 1966. They investigated 13 adult nephrotic patients by means of [51]Cr albumin as a test for gastrointestinal protein loss and made turnover studies with [131]I albumin and [125]I IgG (19). They found no correlation between fractional catabolic rate of albumin and IgG and faecal excretion of [51]Cr which was only slightly elevated in 4 of 13 patients.

The conflicting results may be due to use of different test substances. The validity of the various methods has been discussed in several papers (5, 13, 14, 15, 16, 34, 35). In the present study both albumin turnover studies and [59]Fe labelled iron dextran tests for protein loss were employed. In studies of patients with protein losing gastroenteropathies [59]Fe iron dextran has proven its validity as a suitable test substance which permits a semiquantitative indirect measurement of abnormal protein loss. We have compared faecal [59]Fe clearance and fractional catabolic rate of albumin in the nephrotic children and in 20 children and adults with abnormal gastrointestinal protein loss due to intestinal lymphangiectasia or Crohn's disease. The result is seen in Fig. 1. In patients with protein losing enteropathies a significant positive correlation was present between fractional catabolic rate of albumin and faecal [59]Fe clearance ($r = 0.89$, $p < 0.001$). No such correlation existed in the seven children with nephrotic syndrome. Their fractional catabolic rate of albumin was in several cases greatly elevated despite a normal or only slightly increased [59]Fe clearance. Accordingly their endogenous hypercatabolism of albumin could hardly be ascribed to gastrointestinal protein loss. Thus our results do not agree with those obtained by most other investigators (7, 21, 22, 23, 28, 29, 30) but they do agree with the findings by Jensen *et al* (19) who studied adult nephrotic patients using a valid method for demonstration of gastrointestinal protein loss. The problem of the actual mechanism of protein hypercatabolism remains to be solved.


## SUMMARY

In order to assess the significance of gastrointestinal protein loss as a cause of plasma protein hypercatabolism in patients with nephrotic syndrome, seven nephrotic children were studied simultaneously with radioiodine labelled albumin and a test substance, [59]Fe labelled iron dextran, for evaluation of gastrointestinal protein loss.

All the patients displayed a pronounced hypercatabolism of albumin, which could not be accounted for by urinary protein loss. A slightly increased gastrointestinal protein loss was demonstrated in 4 patients, whereas in 3 patients the faecal loss of macromolecules was within normal limits.

The relation between protein catabolism and faecal loss of macromolecules was compared in the nephrotic children and in 20 patients with protein losing enteropathies. It is concluded that gastrointestinal protein loss is in

Table 3 $^{59}$*Fe labelled iron dextran test in 7 children with nephrotic syndrome*

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| 3 6/12 | F | 0 9 | 1 6 |
| 3 7/12 | F | 1 6 | 2 7 |
| 6 10/12 | M | 1 3 | 1 6 |
| 9 /12 | M | 0 | 0 |
| 11 6/1 | M | 0 5 | 0 5 |
| Normal values | | <1 0 | <0 8 |

tients a normal *D* was found In the remaining 4 it was elevated

Mean life time (*MLT*) varied from 2 to 4 days (normal range 19–33 (25)) It was greatly reduced in all children

Synthetic rate (*S*) amounted to 147–359 mg per kg per day (normal range 120–200 (25)) *S* was normal in 3 cases and elevated in the remaining 4

## DISCUSSION

*It is generally held that the reduction of serum* albumin in the nephrotic syndrome is primarily due to urinary albumin loss However studies by Gitlin *et al* (11) showed that an associated increase of endogenous catabolism of serum albumin and several other plasma proteins in nephrotic children may contribute to the hypoproteinaemia Their results have been confirmed in more recent studies of plasma protein metabolism in adult nephrotic patients (1 2 6 8 9 10 15 17 18 20 22) and in Perheentupa's study from 1967 in children with congenital nephrotic syndrome (31) and in the present study of childhood nephrosis We found a very rapid turnover of serum albumin which could not be accounted for by urinary albumin loss The endogenous fractional catabolic rate was greatly elevated in some patients representing values more than 5 times the normal value Though similar findings have been made in adult nephrotic patients the elevation of the fractional catabolic rate is less pronounced in adults At present we have no explanation of this quantitative difference between children and adult patients

*Distribution ratio mean life time*, total mass and intravascular mass as well as the synthetic rate of albumin in the present series agree well with results obtained in adult nephrotic patients *(6 18 20) The high distribution ratio* means that an abnormally high fraction of the total albumin mass is located intravascularly It agrees well with the fact that the albumin con

Table 4 *Albumin turnover data in 7 children with nephrotic syndrome studied with* $^{131}$*I labelled albumin*

*FDR* – Fractional disappearance rate per cent of intravascular mass disappearing per day from the plasma by way of catabolism and external loss *FCR* – fractional catabolic rate per cent of intravascular mass catabolized per day *D* – distribution ratio per cent of total mass located intravascularly *MLT* – mean life time average life time of an albumin molecule in the organism *S* – Synthetic rate

| Age (yrs) | Sex | Serum albumin (g/100 ml) | *FDR* | *FCR* | *D* | *MLT* Days | *S* g/day | *S* (mg/kg/day) |
|---|---|---|---|---|---|---|---|---|
| 2 1/12 | F | 0 35 | 96 | 42 | 64 | 1 6 | 2 09 | 147 |
| 2 /12 | M | 0 44 | 86 | 61 | 43 | 2 7 | 2 21 | 177 |
| 3 9/12 | F | 0 84 | 67 | 56 | 51 | 2 9 | 3 66 | 240 |
| 3 7/12 | F | 1 12 | 69 | 44 | 68 | 2 1 | 5 39 | 359 |
| 6 10/1 | M | 0 93 | 57 | 42 | 63 | 2 8 | 6 42 | 238 |
| 9 6/12 | M | 0 99 | 53 | 21 | 66 | 2 9 | 7 93 | 283 |
| 11 /12 | M | 0 77 | 46 | 26 | 51 | 4 3 | 6 37 | 152 |
| Normal range | | 3 55–4 99 | | 6 3–11 6 | 41–54 | | | 120–200[a] |

[a] From Krasilnikoff Andersen & Rossing (25)

centration in ascitic and oedema fluid is extremely low in nephrosis It may reflect a decreased capillary permeability or more probably an increased lymphatic flow

The problem remains at which site and by which mechanism the protein hypercatabolism takes place Several studies have been published on gastrointestinal protein loss in nephrotic patients in particular children In some of these investigations an abnormal gastrointestinal protein loss was detected and claimed to account for a significant part of the hypercatabolism (7 21 22 23 28 29 30) Using $^{131}$I polyvinylpyrrolidone Bennhold & Ott in 1962 Nussle *et al* in 1962 Kluthe *et al* in 1963 and Marchal in 1965 demonstrated an increased faecal excretion of $^{131}$I after intravenous injection of the labelled compound in patients with nephrotic syndrome By means of radioiodinated albumin and oral administration of an anion exchange resin Jimenez Diaz *et al* in 1963 and Nussle & Royer in 1964 found an increased faecal excretion of the label in nephrotic patients They concluded that abnormal gastrointestinal protein loss took place In contrast to these results are findings by Jensen *et al* in 1966 They investigated 13 adult nephrotic patients by means of $^{51}$Cr albumin as a test for gastrointestinal protein loss and made turnover studies with $^{131}$I albumin and $^{125}$I IgG (19) They found no correlation between fractional catabolic rate of albumin and IgG and faecal excretion of $^{51}$Cr which was only slightly elevated in 4 of 13 patients

The conflicting results may be due to use of different test substances The validity of the various methods has been discussed in several papers (5 13 14 15 16 34 35) In the present study both albumin turnover studies and $^{59}$Fe labelled iron dextran tests for protein loss were employed In studies of patients with protein losing gastroenteropathies $^{59}$Fe iron dextran has proven its validity as a suitable test substance which permits a semiquantitative indirect measurement of abnormal protein loss We have compared faecal $^{59}$Fe clearance and fractional catabolic rate of albumin in the nephrotic children and in 2 children and adults with abnormal gastrointestinal protein loss due to intestinal lymphangiectasia or Crohn's disease The result is seen in Fig 1 In patients with protein losing enteropathies a significant, positive correlation was present between fractional catabolic rate of albumin and faecal $^{59}$Fe clearance ($r = 0.89$ $p < 0.001$) No such correlation existed in the seven children with nephrotic syndrome Their fractional catabolic rate of albumin was in several cases greatly elevated despite a normal or only slightly increased $^{59}$Fe clearance Accordingly their endogenous hypercatabolism of albumin could hardly be ascribed to gastrointestinal protein loss Thus our results do not agree with those obtained by most other investigators (7 21 22 23 28 29 30) but they do agree with the findings by Jensen *et al* (19) who studied adult nephrotic patients using a valid method for demonstration of gastrointestinal protein loss The problem of the actual mechanism of protein hypercatabolism remains to be solved

## SUMMARY

In order to assess the significance of gastrointestinal protein loss as a cause of plasma protein hypercatabolism in patients with nephrotic syndrome seven nephrotic children were studied simultaneously with radioiodine labelled albumin and a test substance $^{59}$Fe labelled iron dextran for evaluation of gastrointestinal protein loss

All the patients displayed a pronounced hypercatabolism of albumin which could not be accounted for by urinary protein loss A slightly increased gastrointestinal protein loss was demonstrated in 4 patients whereas in 3 patients the faecal loss of macromolecules was within normal limits

The relation between protein catabolism and faecal loss of macromolecules was compared in the nephrotic children and in 20 patients with protein losing enteropathies It is concluded that gastrointestinal protein loss is in-

Table 3 $^{59}$Fe *labelled iron dextran test in 7 children with nephrotic syndrome*

| Age (yrs) | Sex | Faecal $^{59}$Fe output in 4 days of injected dose | Faecal $^{59}$Fe clearance of plasma vol /day |
|---|---|---|---|
| 2 1/12 | F | 0 5 | 0 8 |
| 2 8/12 | M | 1 1 | 1 7 |
| 3 8/12 | F | 0 9 | 1 6 |
| 3 7/12 | F | 1 6 | 2 7 |
| 6 10/12 | M | 1 3 | 1 6 |
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| 9 6/12 | M | 0 99 | 53 | 21 | 66 | 2 9 | 7 93 | 283 |
| 11 [illegible]/12 | M | 0 77 | 46 | 26 | 51 | 4 3 | 6 37 | 152 |
| Normal range | | 3 55–4 99 | | 6 3–11 6[a] | 41–54 | | | 120–200[a] |

[a] From Krasilnikoff Andersen & Rossing (25)

centration in ascitic and oedema fluid is extremely low in nephrosis. It may reflect a decreased capillary permeability or more probably an increased lymphatic flow.

The problem remains at which site and by which mechanism the protein hypercatabolism takes place. Several studies have been published on gastrointestinal protein loss in nephrotic patients, in particular children. In some of these investigations an abnormal gastrointestinal protein loss was detected and claimed to account for a significant part of the hypercatabolism (7, 21, 22, 23, 28, 29, 30). Using $^{131}$I polyvinylpyrrolidone, Beimbold & Ott in 1962, Nusslé *et al.* in 1962, Kluthe *et al.* in 1963 and Marchal in 1965 demonstrated an increased faecal excretion of $^{131}$I after intravenous injection of the labelled compound in patients with nephrotic syndrome. By means of radioiodinated albumin and oral administration of an anion exchange resin Jimenez Diaz *et al.* in 1963 and Nusslé & Royer in 1964 found an increased faecal excretion of the label in nephrotic patients. They concluded that abnormal gastrointestinal protein loss took place. In contrast to these results are findings by Jensen *et al.* in 1966. They investigated 13 adult nephrotic patients by means of $^{51}$Cr albumin as a test for gastrointestinal protein loss and made turnover studies with $^{131}$I albumin and $^{125}$I IgG (19). They found no correlation between fractional catabolic rate of albumin and IgG and faecal excretion of $^{51}$Cr, which was only slightly elevated in 4 of 13 patients.

The conflicting results may be due to use of different test substances. The validity of the various methods has been discussed in several papers (5, 13, 14, 15, 16, 34, 35). In the present study both albumin turnover studies and $^{59}$Fe labelled iron dextran tests for protein loss were employed. In studies of patients with protein losing gastroenteropathies $^{59}$Fe iron dextran has proven its validity as a suitable test substance which permits a semiquantitative indirect measurement of abnormal protein loss. We have compared faecal $^{59}$Fe clearance and fractional catabolic rate of albumin in the nephrotic children and in 20 children and adults with abnormal gastrointestinal protein loss due to intestinal lymphangiectasia or Crohn's disease. The result is seen in Fig. 1. In patients with protein losing enteropathies a significant positive correlation was present between fractional catabolic rate of albumin and faecal $^{59}$Fe clearance ($r = 0.89$, $p < 0.001$). No such correlation existed in the seven children with nephrotic syndrome. Their fractional catabolic rate of albumin was in several cases greatly elevated despite a normal or only slightly increased $^{59}$Fe clearance. Accordingly their endogenous hypercatabolism of albumin could hardly be ascribed to gastrointestinal protein loss. Thus our results do not agree with those obtained by most other investigators (7, 21, 22, 23, 28, 29, 30), but they do agree with the findings by Jensen *et al.* (19), who studied adult nephrotic patients using a valid method for demonstration of gastrointestinal protein loss. The problem of the actual mechanism of protein hypercatabolism remains to be solved.

## SUMMARY

In order to assess the significance of gastrointestinal protein loss as a cause of plasma protein hypercatabolism in patients with nephrotic syndrome, seven nephrotic children were studied simultaneously with radioiodine labelled albumin and a test substance, $^{59}$Fe labelled iron dextran, for evaluation of gastrointestinal protein loss.

All the patients displayed a pronounced hypercatabolism of albumin which could not be accounted for by urinary protein loss. A slightly increased gastrointestinal protein loss was demonstrated in 4 patients, whereas in 3 patients the faecal loss of macromolecules was within normal limits.

The relation between protein catabolism and faecal loss of macromolecules was compared in the nephrotic children and in 20 patients with protein losing enteropathies. It is concluded that gastrointestinal protein loss is in

significant in the nephrotic syndrome, and unable to account for the marked endogenous hypercatabolism of albumin

Albumin synthesis was normal or somewhat increased An abnormally high fraction of the total albumin mass was present in the intra vascular space in 4 of the 7 children

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*Key words* Nephrotic syndrome gastrointestinal protein loss albumin metabolism radioiodine labelled albumin [59]Fe iron dextran

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Table 1 *Age distribution of 95 children with bronchiolitis*

| Age | Prednisolone group | Placebo group | Total |
|---|---|---|---|
| 0–3 months[a] | 21 | 26 | 47 |
| >3–6 months | 15 | 13 | 28 |
| >6 months[b] | 11 | 9 | 20 |
| Total | 47 | 48 | 95 |

$\chi^2 = 0.99$ DF = 2 $0.7 > p > 0.5$
[a] Youngest child was 3 weeks old
[b] Oldest child was 26 months old

Table 2 *Severity of illness in 95 children with bronchiolitis*

| Severity of illness | Prednisolone group | Placebo group | Total |
|---|---|---|---|
| Mild | 14 | 9 | 23 |
| Moderate | 26 | 28 | 54 |
| Severe | 7 | 11 | 18 |
| Total | 47 | 48 | 95 |

$\chi^2 = 2.25$ DF = 2 $0.5 > p > 0.3$

mycin and 50 units of mycostatin per ml. The throat swabs were left to elute for 1 hour at 37°C in the cell culture tubes, then squeezed and removed. The inoculated tubes were rolled at 37°C and were examined daily for cytopathic effects for twenty days. The cell culture medium was changed every third or fourth day and cell cultures showing the presence of syncytia were passaged and the isolated viruses typed by neutralisation with RSV neutralising antiserum. Adenoviruses which were isolated in HEp2 cell cultures were initially identified by electron microscopy and were later typed serologically.

An acute phase serum was obtained from each patient on admission to hospital and convalescent sera were obtained between 23–55 days later. The average interval between acute and convalescent sera was 34 days. Complement fixation tests using the overnight method of Bradstreet & Taylor (3) were performed on the children's sera. Four units of antigen of RSV, influenza virus A, B and C, parainfluenza virus 1 and 3, adenovirus, psittacosis lymphogranuloma, *Coxiella burnetii* and *Mycoplasma pneumoniae* were used.

## RESULTS

Of the hundred patients admitted to the trial there were 36 females and 64 males. There were no deaths. Five patients were later omitted for various reasons. In the placebo group one infant was omitted because of cystic fibrosis and another because of a urinary tract infection. In the prednisolone group two infants who were re-assessed on the morning following admission were found to have bronchopneumonia and coryza respectively. One other child was omitted from this group because of allergy to penicillin.

The age distribution and the severity of illness are shown in Tables 1 and 2 respectively. In either table there was no significant difference between the prednisolone and placebo groups.

The duration of illness was determined from the date of admission until the symptoms and signs disappeared (Table 3) and the total duration of illness from the history of when the symptoms began until they disappeared (Table 4). There was no significant difference in the duration of symptoms and signs on both these assessments between the prednisolone and the placebo groups.

There was no appreciable difference be-

Table 3 *Mean duration of symptoms and signs in hospital in 95 children with bronchiolitis*

| | Prednisolone group | Placebo group |
|---|---|---|
| No. of patients | 47 | 48 |
| Mean duration of symptoms and signs in hospital | 5.89 days | 5.50 days |

Difference in means = 0.39 days
Standard error of difference = 0.466
$t = 0.84$ DF = 93 $0.5 > p > 0.4$

Table 4 *Mean total duration of symptoms and signs in 85* children with bronchiolitis*

| | Prednisolone group | Placebo group |
|---|---|---|
| No. of patients | 43 | 42 |
| Mean total duration of symptoms and signs | 9.69 | 9.19 |

Difference in means = 0.50 days
Standard error of difference 0.66
$t = 0.61$ DF = 83 $0.6 > p > 0.5$
* In an additional 10 patients the history was unreliable

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# A DOUBLE BLIND TRIAL OF PREDNISOLONE IN EPIDEMIC BRONCHIOLITIS DUE TO RESPIRATORY SYNCYTIAL VIRUS

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Epidemics of bronchiolitis occur in most large cities in Britain during the winter months (1 12, 18) It was shown by Chanock *et al* (4) that respiratory syncytial virus (RSV) was of major importance in the causation of bronchiolitis and pneumonia in infants and young children

In a disease which affects such large numbers of children it is important to decide which forms of therapy are of value It has been shown that antibiotics do not influence the course of bronchiolitis (9) Corticosteroids have been used in the treatment of bronchiolitis (6 7 8) but their value in influencing the course of the disease remains controversial and the present double blind trial was designed to test the efficacy of prednisolone in the treatment of bronchiolitis

## MATERIAL

An epidemic of bronchiolitus began in Belfast N Ireland during the first week of November 1966 The number of admissions to hospitals increased to a peak during the weeks ending 4th and 11th December 1966 and the epidemic ended on the last week of January 1967 During this period three hundred and ninety four children between the ages of 0–2 years required admission to Belfast hospitals with acute respiratory illnesses

*Patients*

One hundred infants were admitted to the trial between 6th December 1966 and 8th January 1967 The criteria for selection were coryza increased respiratory rate paroxysmal cough and expiratory wheeze The progress of each child was recorded daily on a chart which listed temperature respiratory rate the presence of cyanosis the use of accessory muscles of respiration sub costal recession cough expiratory wheeze and adventitious sounds in the chest

All the children were given Ampicillin as it was thought unwise to treat infants with a severe respiratory infection with prednisolone without antibiotic cover Prednisolone or an apparently identical placebo was given according to a randomised code Prednisolone was given in decreasing dosage 15 mg was administered on the first day 10 mg on the second and third days 5 mg on the fourth and fifth days and 2 5 mg on the sixth and seventh days Except for a few of the mildest cases all were treated in an oxygen tent with aerosol water vapour until their condition improved

The children were classified into three groups according to the severity of their illness Mildly ill cases had pyrexia increased respiratory rate expiratory wheeze and crepitations in the lungs Moderately ill cases had in addition to the above cyanosis which was rapidly relieved sub costal recession and the accessory muscles of respiration were used In the severely ill cases the above signs were more marked and cyanosis persisted for more than 24 hours

## METHODS

*Virology*

Two throat swabs were obtained from each patient within twenty four hours of admission to hospital Each throat swab was inoculated directly (12) into a tube containing HEp2 cell culture in Eagles medium with either 4 % rabbit serum (Burroughs Wellcome brand no 1) or 1 % foetal calf serum (Flow Laboratories Rockville Md ) The cell culture medium contained 100 units of penicillin 100 mcg of strepto

clinical course of epidemic bronchiolitis Dabbous *et al* (5) also showed that prednisolone was of no value in a smaller trial in children with bronchiolitis of unknown aetiology Treatment of bronchiolitis depends essentially on humidified oxygen adequate sedation and hydration there is no specific drug which will take the place of good nursing care

The general effect of adrenocortical hormones on virus infections and immunity in experimental animals is not only to increase the susceptibility to infection and viral multiplication but also to diminish the antibody response (16) There was no evidence in this trial that prednisolone treatment of the patients affected the antibody response Since the trial was carried out during an epidemic of bronchiolitis and RSV infection was confirmed virologically in 84 of patients it was assumed that almost all the children in the trial were infected with this virus Two authors of recent paediatric textbooks (13 15) while not convinced of the benefits of corticosteroids do use them in very ill patients In the dosage used in this trial prednisolone had no beneficial or harmful effects on the course of the disease in severely ill children

Twenty one children aged three months or younger had RSV complement fixing antibody in their acute phase serum and only 14 developed an antibody response to RSV infection This implies that the mothers of these children had RSV antibody in their sera in sufficient titre to be detectable after transplacental passage The presence of neutralizing as well as complement fixing antibodies to RSV in umbilical cord blood in children under six months was shown by Hambling (11) When no detectable complement fixing maternal RSV antibody was present in the acute phase serum there was a 91 serological response to infection These facts suggest that the presence of low levels of maternal antibody while not preventing RSV infection and bronchiolitis does prevent an active complement fixing antibody response in about 77 of children of this age group In a study of children aged 5–11 years Potash *et al* (17) found that the serological response to RSV vaccine was poor in those children who had pre-existing antibody before vaccination Infection with RSV has been shown to occur in the presence of antibody in the serum of adults (14) and of children (2 10) However resistance to infection with some respiratory viruses may depend more on the antibody present in the respiratory tract secretions rather than on the serum antibody titre (19)

The eight children who were infected with both RSV and adenoviruses spent on average, two days longer in hospital and their illness was more than three days longer overall which suggests that double infections can cause a more severe illness than RSV infection alone

## SUMMARY

A double blind trial of prednisolone treatment was carried out on 95 children with clinical evidence of epidemic bronchiolitis The trial showed that there was no difference between the prednisolone and the placebo group in the duration of symptoms and signs

Eighty four per cent of children were shown to be infected with respiratory syncytial virus Prednisolone in the dosage used did not affect the antibody response to infection

The presence of maternal antibody to respiratory syncytial virus while not preventing infection reduced the number of active antibody responses in children aged 3 months or younger

Eight children had a double infection with respiratory syncytial virus and adenovirus which lengthened their average stay in hospital by two days and the overall duration of illness by more than three days

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tween the medium containing 4 rabbit serum and the medium containing 1 foetal calf serum in the RSV isolation rate or the day on which virus cytopathic effects appeared Throat swabs were obtained from 93 children Both throat swabs from 11 patients were contaminated with yeasts or fungi and of the 82 patients remaining RSV was isolated from one or both throat swabs of 53 patients—an isolation rate of 65 The RSV isolation rate in the prednisolone treatment group was 67 while in the placebo treatment group it was 60

Acute and convalescent sera were obtained from 86 patients The RSV antibody responses in the prednisolone and placebo groups are shown in Table 5 Sixty four patients (74 ) had a four-fold or greater rise in RSV antibody and this included two patients from whom adenovirus type 2 was isolated It will be seen that prednisolone in the dosage used did not prevent or depress RSV antibody formation when compared with the placebo group

Ninety three patients in the trial had suitable specimens taken either for virus isolation or serology or both and a diagnosis of RSV infection was made in 78 patients by either virus isolation or a four fold or greater rise in RSV antibody which gives an overall diagnostic rate of 84 •

The RSV complement fixing antibody data and duration of illness in 43 children aged 3 months or younger is shown in Table 6 The results show that RSV complement fixing antibody was present in the acute phase sera of 21 children but only 14 •. of them developed a rising titre of antibody although RSV was isolated from 71 of them Twenty two children did not have RSV complement fixing antibody at a 1 4 dilution in their acute phase and 91 of them developed a rising titre of antibody There was no appreciable difference in the average duration of illness in the two groups

Table 5 *The RSV antibody responses in the prednisolone and placebo treatment groups*

| Rise in RSV antibody titre | Number of patients | | |
|---|---|---|---|
| | Prednisolone group | Placebo group | Total |
| <4 fold | 8 | 14 | 22 |
| 4 fold | 11 | 11 | 22 |
| 8 fold | 6 | 8 | 14 |
| 16 fold | 6 | 7 | 13 |
| 32 fold | 5 | 3 | 8 |
| 64 fold | 3 | 2 | 5 |
| 128 fold | 1 | 0 | 1 |
| >128 fold | 1 | 0 | 1 |
| Total | 41 | 45 | 86 |

Table 6 *RSV antibody data and duration of illness in 43 children aged 3 months or younger*

| Patients | RSV complement fixing antibody in acute phase serum (1 4 dilution) Present | Absent |
|---|---|---|
| No tested | 21 | 22 |
| No showing 4 fold or greater rise in titre | 3 (14 ) | 20 (91 %) |
| Percentage from whom RSV was isolated | 71 | 62 |
| Mean duration of illness in hospital | 5 4 days | 6 4 days |
| Mean total duration of illness | 9 4 days | 9 8 days |

Eight children had a double infection with RSV and adenoviruses as shown by either virus isolation or a four fold or greater rise in antibody titre The average duration of illness in hospital for these eight children was 7 5 days and the average total duration of their illness was 13 days (see Tables 3 and 4)

A greater than four fold rise of antibody to parainfluenza virus type I developed in one patient and to psittacosis lymphogranuloma agents in another patient Virus was not isolated from either patient and antibody to the other antigens did not develop Three other children had parainfluenza type I antibody and seven children had parainfluenza type 3 antibody in their sera but in all these children the antibody titres either fell or remained static

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clinical course of epidemic bronchiolitis Dabbous et al (5) also showed that prednisolone was of no value in a smaller trial in children with bronchiolitis of unknown aetiology Treatment of bronchiolitis depends essentially on humidified oxygen adequate sedation and hydration there is no specific drug which will take the place of good nursing care

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# ERYTHROPOIETIN LEVELS IN CORD BLOOD OF CONTROL INFANTS AND INFANTS WITH RESPIRATORY DISTRESS SYNDROME

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Prenatal asphyxia may precede development of the respiratory distress syndrome (hyaline membrane disease) in the premature infant The evidence for this is indirect It is based upon the increased frequency of conditions which might cause fetal asphyxia (caesarian section maternal bleeding or maternal diabetes) (1) and upon the condition of the infant at birth as judged by the Apgar score (10)

The present study was undertaken to document more directly and define the role of anoxia in the respiratory distress syndrome using cord blood levels of erythropoietin (ESF) This should be possible because ESF levels are increased in the cord blood of infants with certain pregnancy complications related to asphyxia and because normal premature infants are able to produce ESF (5 8)

## CLINICAL MATERIAL

Cord blood specimens were collected from eleven premature infants with the respiratory distress syndrome nine premature infants without respiratory distress and five full term infants without findings of fetal malnutrition intrauterine distress or abnormal hemolysis Specimens of cord blood were collected in heparinized tubes and the plasma separated and stored at −20 until assayed for ESF The severity of the respiratory distress was judged by the scale illustrated in Table 1

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## LABORATORY METHODS

Erythropoietin levels were determined by measuring the $^{59}$Fe incorporation into red cells of the hypoxia induced polycythemic mouse after injection of cord plasma CF 1 female mice weighing 20–30 g were used and hypoxia was produced in an altitude chamber at 0 4 atmospheres Two procedures were used The first modified from Weintraub (13) utilized 110 hours of hypoxia 8 hours/day for 14 days On days 4 and 5 after removal from the altitude chamber 0 5 cc of plasma was injected subcutaneously (s c) On day 6 1 μc $^{59}$Fe in 1 cc normal saline was injected intravenously (tail vein) On day 9 the 72 hours % $^{59}$Fe incorporation was determined The second procedure that of Cotterra (3) utilized 200 hours of hypoxia over a 10-day period The day the animals were removed from the chamber was day 0 of the assay On days 4 and 5 1 cc of plasma was injected s c The $^{59}$Fe was injected on day 6 and the 48 hour % $^{59}$Fe incorporation determined on day 8 In calculation of the % $^{59}$Fe incorporation total blood volume was estimated to be 7 3% of body weight Uninjected mice served as controls with both procedures

With both procedures standard human urinary ESF was used to establish response curves and all determinations were expressed as units of ESF per ml of plasma All values above 0 05 units (u)/ml are reported to the nearest 0 1 unit Values less than 0 05 u/ml and significantly higher ($p < .05$) than uninjected controls (zero) are reported as <0 05 u/ml

## RESULTS

Weights of the 20 premature infants varied from 1100 to 2400 grams Lengths of gestation varied from 27 weeks to 39 weeks (last menstrual period) (Table 2) ESF levels were

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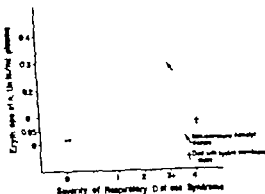



Fig 1 The relationship between cord blood erythropoietin levels and the severity of the respiratory distress syndrome

intrauterine hypoxia Both full term and premature infants are capable of producing ESF when stressed by either anemic or hypoxic hypoxia and normal full term infants have significantly higher levels of ESF than normal premature infants Furthermore elevated cord blood levels of ESF are found in infants born of complicated pregnancies thought to be associated with intrauterine hypoxia i e pre eclampsia post maturity and maternal diabetes (15)

These findings are consistent with the findings of reduced % $O_2$ saturation in the umbilical artery of infants of preeclamptic and diabetic mothers (2) This suggested that a study of cord blood levels of erythropoietin might confirm the importance of anoxia in the genesis of the respiratory distress syndrome

Increased levels of ESF can be demonstrated in the adult human subjected to relative hypoxia at high altitude This response is maximal after 24 hours of continued hypoxia Following several days of continuous hypoxia the ESF levels fall to undetectable levels (12) In addition the elevated levels of ESF found in anemic adults disappear within 12–24 hours after blood transfusion (9) Elevated levels of ESF in cord blood are also gone by the end of the first day of life (7)

Therefore it would be expected that an hypoxic episode shorter than 8–12 hours in duration longer than 2–3 days in duration or terminated more than 12–24 hours before birth might not be reflected by an elevated level of ESF in the cord blood However an acute hypoxic episode of several hours duration occurring within the 12–24 hours just before delivery might be detectable by ESF assay of cord blood

The present study demonstrates no difference in cord blood ESF between premature infants with and without the Respiratory Distress Syndrome This suggests that either intrauterine hypoxia plays no role in the genesis of the respiratory distress syndrome or that the hypoxia is (1) of short duration (2) of relatively long duration or (3) remote with respect to the time of birth

It is significant that premature infants with or without the respiratory distress syndrome had significantly lower levels of ESF than did a small group of full term infants This confirms the findings of Finne who found significantly lower levels of ESF in normal premature infants when compared to full term infants (5) However premature infants with erythroblastosis seem capable of responding to anemic anoxia with levels of ESF in amniotic fluid proportional to the degree of anemia (4 6) This suggests that hypoxia before birth is a more common feature of the term delivery than of the premature delivery

## SUMMARY

Erythropoietin levels (ESF) were measured in premature infants with and without the respiratory distress syndrome in an effort to define the role of intrauterine hypoxia in the genesis of the disease No difference in levels could be detected between infants with and without the respiratory distress syndrome This suggests that either intrauterine hypoxia plays no role in the genesis of the respiratory distress syndrome or that the hypoxia is (1) of short duration (2) of relatively long duration or (3) remote with respect to the time of birth The higher levels found in full term infants sug

Table 1 *Severity of respiratory distress syndrome*

| | |
|---|---|
| 0 | No respiratory distress |
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| 1+ | Mild disease no chronic cyanosis no $O_2$ required Respiratory signs persist for more than 24 hours |
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elevated in 5 of 11 premature infants with respiratory distress syndrome and 3 of the 9 premature infants without respiratory distress an insignificant difference between these two groups of infants (Fig 1) The median level of ESF of the 20 premature infants was < 0 05 u/ml In contrast, the median level in the 5 full term infants was 0 3 u/ml ESF levels greater than 0 05 were present in all 5 full term infants and were significantly more frequent than in the premature infants ($\chi$ —with Yates correction) = 5 367 $p$ = 0 022)

## DISCUSSION

The evidence that intrauterine asphyxia plays a role in the genesis of the respiratory distress syndrome is circumstantial and presumptive Clinical evidence is based upon increased incidence of conditions suggested to cause fetal asphyxia (1) Experimental evidence in human infants is based upon cord blood arterial and venous pH and blood gas studies which are influenced by events just preceding delivery (11) Finne has proposed using cord blood levels of ESF as a potential means of assessing

Table 2 *Cord blood erythropoietin levels*

| Birth weight (G) | Race (negro = N caucasian = C) | Sex | Gestation (weeks) | R D S | E S F (u/ml) | Mice (no ) | Other comments |
|---|---|---|---|---|---|---|---|
| 2440 | N | M | 39 1/7 | 4+ | 0 1 | 2 | Infant of a diabetic mother died with hyaline membranes with intracranial hemorrhage |
| 1077 | N | M | — | 3+ | 0 0 | 1 | |
| 2183 | N | M | 33 1/7 | 3+ | 0 3 | 3 | Non isoimmune hemolytic disease not anemic at birth |
| 1650 | N | M | — | 2+ | 0 0 | 3 | |
| 1310 | N | M | 31 0/7 | 2+ | 0 2 | 2 | |
| 1190 | N | M | 26 5/7 | 2+ | 0 0 | 1 | |
| 1910 | N | F | 35 0/7 | 1+ | 0 0 | 2 | |
| 1750 | C | M | 35 3/7 | ∓ | 0 0 | 2 | |
| 1900 | C | M | 39 2/7 | ∓ | 0 2 | 1 | |
| 2160 | N | F | 38 1/7 | ∓ | 0 0 | 2 | |
| 1770 | C | F | 31 6/7 | ∓ | <0 05 | 2 | |
| 2340 | N | F | 39 3/7 | 0 | 0 0 | 2 | |
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| 2120 | C | F | 38 4/7 | 0 | 0 0 | 2 | |
| 1880 | N | F | 31 0/7 | 0 | 0 2 | 3 | |
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| 1650 | N | F | — | 0 | 0 0 | 2 | |
| 1590 | N | M | 36 2/7 | 0 | 0 0 | 2 | |
| 1418 | N | F | 34 0/7 | 0 | 0 1 | 1 | |
| 3360 | N | M | 36 5/7 | 0 | 0 2 | 3 | Normal full term |
| 3260 | N | M | 45 3/7 | 0 | 0 1 | 4 | Normal full term |
| 3540 | C | M | 40 6/7 | 0 | 0 4 | 3 | Normal full term |
| 2960 | N | M | 32 0/7 | 0 | 0 3 | 1 | Normal full term |
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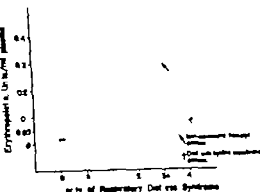


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| 1650 | N | M | — | 2+ | 0 0 | 3 | |
| 1310 | N | M | 31 0/7 | 2+ | 0 2 | 2 | |
| 1190 | N | M | 26 5/7 | 2+ | 0 0 | 1 | |
| 1910 | N | F | 35 0/7 | 1+ | 0 0 | 2 | |
| 1750 | C | M | 35 3/7 | ∓ | 0 0 | 2 | |
| 1900 | C | M | 39 2/7 | ∓ | 0 2 | 1 | |
| 2160 | N | F | 38 1/7 | ∓ | 0 0 | 2 | |
| 1770 | C | F | 31 6/7 | ∓ | <0 05 | 2 | |
| 2340 | N | F | 39 3/7 | 0 | 0 0 | 2 | |
| 2240 | N | F | 38 2/7 | 0 | 0 0 | 1 | |
| 2120 | C | F | 38 4/7 | 0 | 0 0 | 2 | |
| 1880 | N | F | 31 0/7 | 0 | 0 2 | 3 | |
| 1804 | N | M | 36 1/7 | 0 | <0 05 | 2 | |
| 1600 | N | F | 33 0/7 | 0 | 0 0 | 1 | |
| 1650 | N | F | — | 0 | 0 0 | 2 | |
| 1590 | N | M | 36 2/7 | 0 | 0 0 | 2 | |
| 1418 | N | F | 34 0/7 | 0 | 0 1 | 1 | |
| 3360 | N | M | 36 5/7 | 0 | 0 2 | 3 | Normal full term |
| 3260 | N | M | 45 3/7 | 0 | 0 1 | 4 | Normal full term |
| 3540 | C | M | 40 6/7 | 0 | 0 4 | 3 | Normal full term |
| 2960 | N | M | 32 0/7 | 0 | 0 3 | 1 | Normal full term |
| 2870 | C | M | 39 0/7 | 0 | 0 4 | 4 | Normal full term |

*Acta Pædiat Scand 58* 125–132, 1969

# PAEDIATRIC EDUCATION

## *The Place of 'Social Paediatrics' in Undergraduate Teaching*

F J W MILLER

*From the Department of Child Health University of Newcastle upon Tyne England*

My choice of subject the teaching of social (or community) paediatrics to undergraduate students has been determined by my belief that the practice of medicine is more than a study of human biology—it is basically a social service Since children are particularly susceptible to environmental influences of all kinds the teaching of paediatrics has a social component which must be understood by teachers and conveyed to students

Perhaps you may think it strange that I should make such a statement for in Sweden, throughout the whole history of paediatrics from the establishment of the first chair in 1845 the subject has been broadly based in the community as well as in the hospital Indeed if I understand the situation correctly professors have always had responsibility for activities concerning the care of children outside their university departments and their hospitals and thus have always had effective community contacts In this country therefore paediatrics had the good fortune to become established when the needs for prevention and community care were perhaps more obvious than today and the distractions of technical procedures and investigations so fascinating intellectually and so highly rewarded in status were not so tempting to your physicians The modern dilemma of techniques and ethics had not then been posed

But now most hospital doctors never see their patients at home and medical students and junior doctors find it difficult to be constantly aware of all the social variables and are apt to think of the patient only as he appears in a hospital bed

Furthermore in 'developing' countries we see hospitals reproducing the designs of those in advanced and wealthy states and their young men travelling abroad learn techniques which can only be applied in a setting which has little relevance to the mass of their people Being trained in a certain way many are unable later to adapt to the needs of their local situation And this is the heart of the matter—that paediatrics cannot be taught or practised without reference to the social context of the community concerned My purpose is to show the conditions required for that kind of teaching.

Before we consider the content of teaching, it is necessary to do three things set limits to the lecture define social paediatrics indicate the functions of a university department

1 First I intend to speak only about those aspects of social paediatrics—teaching and research—which concern a university department and only in respect of undergraduate education I am not here concerned with other activities which may equally well be called

Carl Gyllenswärd lecture held in Stockholm May 9 1968

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The paths chosen for study will vary from department to department and can concern clinical laboratory or social studies so that each department in a country might have its different and characteristic outlook

If these four points can be accepted as describing the functions of a university department, we should next consider how they affect *teaching* First, the traditional division between curative and preventive medicine so often implicit in teaching and explicit in organisation disappears and is replaced by a concern for both aspects in the individual and the community Furthermore, we can accept that teaching and practice must be related to the needs of the particular community in which the university is situated The aim is to equip the student with the ability to look not only at an incident of disease, but to the underlying and determining causes the variables are the economics and the epidemiological and cultural characteristics of the local community

We can see also that social attitudes affecting students and staff are made manifest in three areas—*clinical practice, teaching* and research

## SOCIAL ASPECTS OF CURRENT PAEDIATRIC TEACHING

Since local needs vary and also because medical education varies from country to country it is likely that the social emphasis and content of teaching will also vary Despite this variation if we know how social paediatrics is *taught in departments in different* parts of the world we might be able to deduce certain general requirements necessary for effective teaching

This I have tried to do for a number of countries and the task has been lightened very considerably by a series of reports on paediatric education which appearing in the last 12 years, reflect a growing concern that the quality of all teaching should improve and that it should be directed towards making physicians *aware of and anxious to meet*, the needs of the community they serve

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During the final year, one month residency in paediatrics is mandatory. This is practical ward experience and again, as in the previous stage, emphasis is placed on the child as a social being to whom the hospital is no more than an incident. In this month the students visit welfare centres and again see mothers and infants outside the hospital setting and receive instruction in infant feeding and prophylactic immunisation.

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the community In addition he brings the department into association with those of public health or social medicine and with the social services for children he establishes necessary and relevant data regarding his own community and pursues enquiry appropriate and pertinent to his local situation Thus an intimate knowledge of local causes of mortality and morbidity is acquired together with effects of any local problems and characteristics of the particular community This was the basic reason for the thousand family study in Newcastle (11 17) the study of organisation for the care of premature children and now although initiated in the Department of Obstetrics the Newcastle maternity survey and its sequential study of growth all these have been joint efforts between university departments and the local health authorities and can be properly described as social obstetrics and paediatrics

Jackson (9) who has made the most recent survey of undergraduate peadiatric education in the united Kingdom gave the declared aims of paediatric teachers to be

1 To teach the importance of family background and the art of human relationship
2 To teach the recognition and handling of the well and sick child
3 To emphasize the importance of growth and development and the disturbances caused by genetic and environmental factors
4 Factual teaching about specific diseases

His review however does not indicate how the third—and fundamental—point is taught except to say that all schools arrange for students to attend infant welfare clinics or neonatal follow up clinics Yet he estimates that only half of all medical schools provide regular opportunities for domiciliary visits by students Most do arrange for their students to visit special institutions such as mental deficiency hospitals, day nurseries schools for handicapped children

The *Report of the Royal Commission on Medical Education* has just been published (14) Unfortunately no member of the commission was a paediatrician and the report has little to say about paediatrics as a subject But it does advise that the student is taken out of hospital— later the student should study the child in his home and school environment and be made aware of the services provided by local authorities and others for the care of children He should be made familiar with the growth and development of the normal child and with immunisation procedures "

### *France*

In France in the last 15 years Debre has done much to advance paediatric education and in Lisbon he listed aspects of preventive paediatrics which must be taught (5) But he works in Paris in the International Childrens Centre, not in a university and he does not teach undergraduates

As far as I can discover from correspondence the subject in university departments (except in a few centres, such as Nancy) has met considerable difficulties and there is little emphasis on social aspects of paediatrics during undergraduate teaching

### *Sweden*

Sjolin in 1965 (16) said of Sweden paediatric teaching is more comprehensive than 20 years ago but much attention is given to rare diseases while minor conditions are included far less than would correspond to their frequency home visiting has had to be abandoned

In spite of interest in preventive and social paediatrics, students do not take an active part in that type of work it is particularly desirable that students in the future should get better opportunities for practical work among the sick and healthy children

In Sweden as in other advanced countries the care of early and adolescent emotional changes and difficulties and social and educational difficulties in the long term supervision of diabetic and handicapped children are indeed important

### *Turkey*

During the Lisbon meeting also a speaker from Turkey stated with engaging frankness that the young men of his country preferred to be engaged in cardiological techniques than to take up social paediatrics

### *Russia and associated countries*

In the health services of the socialist" countries the care of children is practised by paediatricians responsible for both curative and preventive medicine Logically therefore teaching is also specialised and the student decides very early whether to take paediatrics before basic training is complete Most of the paediatricians are women and the rise of this type of education has been accompanied by a great improvement in vital statistics and in the health of children (21)

### *North America*

This great country has still a fragmented health service and in major cities with their mobile nuclear suburban families the paediatrician also takes the place of relatives though he practises largely in his office and does few home visits In the same cities large under doctored slum areas are dependent upon hospitals for their care At the present time a distinguished paediatrician can say quite bluntly that both the training and distribution of doctors are wrong (10)

Hospitals as they attempt to give students teaching in family medicine for community paediatrics extend this activity into the community Essentially they teach by giving a student responsibility for supervision of one or more families and from that point build up programmes often elective in community paediatric practice or research (1 7) In Rochester one of the most community minded departments of the country during paediatric postings there are regular conferences in which emotional and social problems are discussed topics such as the battered child the dying child adoption early rearing practices and so on Programmes involving work in community schemes concerning the care of indigent families continue into residency programmes And finally there are projects in social and community care available for students during elective periods

### *Canada*

In Vancouver a programme exists employing teachers with dual training and appointments in social medicine and paediatrics (13)

*In his first* year the student is assigned a family with one or more children which he must visit and care for clinicians guide and instruct the students in regular meetings and seminars During part of the second year he works for a short period in a centre developed to care for the children of university students In his third and fourth years the student conducts an "office practice in outpatients under the guidance of medical staff and works in the wards He also has the opportunity to see the work of various social agencies Each student must write a project and many choose one with a social paediatric content

### *Australia*

In Australia a high proportion of the population is gathered in aggregations in and about the five major cities In Sydney Clements has evolved a course in social paediatrics which he equates with community child health (3 4) Working in a sophisticated community he realised that the adverse factors—infection and malnutrition—are minimal risks compared with the social and emotional strain of family life The social aspects of clinical paediatrics are stressed during ward and outpatient teaching but Clements believes it is essential that students should see children at home

Accepting the basic difficulties he thinks that these concepts can be taught adequately only as a case discussion using information actively collected by the students from chosen mothers and children in welfare centres This concept has been organized into a course on social paediatrics which occupies 6 students for 4 weeks In the first week which is compulsory he explains the significance of home care Sub-

sequent meetings are voluntary, the great majority of students attend, and in the second week the students visit a kindergarten Each pair is allocated a child whose behaviour is watched and noted the two students then visit the child's home and meet and talk with his mother Finally in the fourth week, they present and describe the home background with their assessment Discussion then takes place in the presence of social workers and teachers During the same term the whole group of some 18–20 students has a weekly seminar in social paediatrics dealing with subjects such as accident prevention (3)

*Africa*

Coming to Africa in Ibadan Nigeria, the Department of Paediatrics is making considerable progress the students have two periods of paediatrics each of two months separated by two months of rural community medicine when they see much paediatrics also During both postings students visit the homes of patients who have been in the wards and with the health visitor give "on the spot" advice They also report back to the department and situations are discussed in weekly seminars covering aspects of social paediatrics such as nutrition local habits and beliefs and in this way students experiences are related to the wider needs and problems of the community (8)

*Dakar*

In Dakar in a programme developed by Senecal (15) the student must visit a family once a week reporting change and watching his family through illness and stress Once a month he attends a meeting of physicians for practice in team work Then he has courses of instruction in the paediatric wards and in the M C H centre and, finally and most originally he goes to a rural centre serving about 45 000 people

*India*

In India paediatrics is a young subject, not yet everywhere independent of medicine, the departments are thronged with partients and student/staff ratio is often very high 100 medical colleges exist where 20 did in 1947 Under these conditions it is not surprising that social paediatrics has not yet developed in many departments only in a few schools, did I see undergraduates in paediatrics get out of hospital into peripheral clinics Lack of social services and the poverty and illiteracy combine to make social education very difficult yet it is essential that health education must combine with curative medicine if medicine is to play its proper role in the development of the country

Despite difficulties, it is evident throughout the world that awareness of the social implication of paediatrics is growing and the days when the social content of medicine was not considered—the word does not appear in the index of Flexner's report on medical education (6) or Newman's *Evolution of Medical Education in England in the 19th Century* (12)—have gone It is true the need is often only acknowledged almost as an afterthought, for example in the Rock Carling Lecture of 1965 a distinguished surgeon discussing medical education could say rather limply Time would have to be found for instruction in public health and social medicine which are becoming increasingly important (19) But the social component can no longer be ignored

Having looked briefly at these social aspects of paediatric teaching in different countries, can we now detect features common to each and thus attempt to set out the basic requirements for teaching of the subject from a university department?

First we must acknowledge that teaching of this kind is difficult its effects are not necessarily immediate and it is difficult to assess Not all students can understand or appreciate its relevance Youth usually demands a quick return for time and effort Teaching must therefore be carefully designed, as far as possible woven into the fabric of clinical instruction directly concerned with patients and with active projects for these rather than the passive reception of information, constitute real learning

Secondly senior teachers must have manifest interest for students know what a department values and they quickly detect the difference between real concern and lip service just as they sense the intra-hospital *prestige* value of the different disciplines

*Thirdly* students must be divided into small groups and the staff/student ratio is necessarily low Social teaching is most effective when it has behind it some organized social services not surprisingly health social organization and the development of complex educational forms usually run parallel with growing wealth and with increasing standards of general education And even in some European countries the number of students *is so great that* small group teaching seems impossible

Despite apparently slow progress successful teaching is taking place in many parts of the world where there is increasing awareness of the true purpose of medicine and the need to understand the sociology of medical care in communities of different types and varying degrees of complexity

Our subject is certainly too big for the time and space at our disposal so let me conclude by asking you to consider as did the writer of the *Book of Proverbs* (ix i)—Wisdom builded a house she hath hewn out her seven pillars—the seven pillars of the teaching of social paediatrics

These in my judgement and experience are

1 Teachers must show social concern
2 Senior as well as junior teachers must be active in social teaching
3 The university department must have roots in the local community
4 The student is taken outside the hospital for practical teaching and introduced to family situations
5 The central theme of teaching is Development and its determinants individual and community
6 Teaching programmes have relevance to local situations and the role of the practice of medicine and paediatrics
7 A programme of community enquiry provides data to clarify problems and to teach with factual knowledge

These seven *characteristics* seem to me to be the general principles governing all programmes of social paediatrics they integrate with curative clinical paediatrics on one hand and with social medicine and obstetrics on the other Medicine in the end despite all our technical research today is more than an understanding of human biology—*it is* in the final analysis a personal and community service

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Acta Pædiat Scand 58 133–140 1969

# ACID BASE AND ELECTROLYTE BALANCE IN NEWBORN INFANTS OF DIABETIC MOTHERS

BERTIL THALME and LARS ENGSTRÖM

*From the Department of Pediatrics and the Department of Obstetrics and Gynecology Karolinska Hospital and the Department of Clinical Biochemistry Royal Veterinary College Stockholm Sweden*

The fetal development when the mother has diabetes mellitus is affected by the altered maternal environment The deranged metabolism influences the acid base and electrolyte status of the mother The relationship between components in maternal and fetal blood has previously been documented (3 12 13 19 22) and recently it has been shown that changes in the acid base and electrolyte balance of healthy pregnant women at midterm are reflected in corresponding changes in the fetus (18)

The acid base status of infants of diabetic mothers has been claimed either to differ significantly from that of normal infants (4 5) or to be similar to that of normal infants (10)

The plasma electrolytes have also been reported either to be of normal or abnormal concentration Normal levels have been reported for potassium sodium and chloride (14 21) while abnormal levels have been found for calcium and phosphate (20)

In most of the studies mentioned above the acid base and electrolyte status of infants to diabetic mothers delivered by caesarean section have been related to that of newborn infants to healthy mothers after vaginal delivery In the present investigation both the groups of infants to diabetic mothers and the control group of infants to healthy mothers were delivered by caesarean section

The aim of the study was to determine to what degree the acid base and electrolyte situation in infants of mothers with well-controlled diabetes differ from the values obtained in infants of healthy mothers The values obtained in the two groups of infants were related to maternal values

## MATERIAL AND METHODS

### *Newborn infants*

*Eight infants of diabetic mothers (IDM)* were studied The infant were delivered by caesarean section after a duration of pregnancy of 37 to 38 weeks Birth weights ranged from 2000 to 3700 g Except for one infant (no 8) these weights were all between the 10th and 90th percentile for gestational age according to the weight chart of Engstrom & S erky (2) Infant no 8 was small for gestational age due to maternal toxaemia The condition of the infants at birth was good in all cases except in two (nos 4 and 5) Infant no 4 did not breathe spontaneously until 1 / minutes after birth and infant no 5 had an initial Apgar core of 7 At 24 hours after birth and throughout the first week, the condition of six of the infants was satisfactory Two (nos 2 and 7) developed hyaline membrane di ea e and we e treated in a respirator Case no 2 survived af er 4 days treatment while ca e no 7 died af er ⌊ days

*I fants of healthy mothers (IHM)* The con rol group comprised eight infants of healthy mothers also delivered by caesarean section The duration of pregnancy was 38 to 40 weeks and birth weights ranged from 2790 to 4340 g In the control group o e infant (no 7) had a weight above the 90th percentile The condition of these newborn infan s was good at birth, at 24 hours and during the first week of life

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Table 1 *The effects of diabetes on different chemical constituents in maternal venous plasma*

Comparison between the values of the control and the diabetes study

| Analysis | $\bar{x}_{control}$ | | $\bar{x}_{diabetes}$ | | Difference | Significance |
|---|---|---|---|---|---|---|
| pH | 7 551 | (7) | 7 479 | (8) | 0 072 | |
| $pCO_2$ (mm Hg) | 18 9 | (7) | 22 9 | (8) | 4 0 | — |
| $BE_p$ (mEq/l) | −6 9 | (7) | −7 2 | (8) | 0 3 | — |
| $K^+$ (mEq/l) | 4 16 | (7) | 3 88 | (8) | 0 28 | — |
| $Na^+$ (mEq/l) | 135 1 | (7) | 131 6 | (8) | 3 5 | — |
| $Cl^-$ (mEq/l) | 108 3 | (7) | 103 3 | (7) | 5 0 | — |
| $HCO_3^-$ (mEq/l) | 16 1 | (7) | 16 2 | (8) | 0 1 | — |
| $Ca^{++}$ (mEq/l) | 5 10 | (6) | 4 50 | (7) | 0 60 | — |
| Total protein (mEq/l) | 16 6 | (6) | 15 4 | (8) | 1 2 | — |
| $HPO_4^{--}$ (mM/l) | 0 73 | (6) | 1 07 | (7) | 0 34 | * |
| Glucose (mg/100 ml) | 117 6 | (5) | 270 2 | (6) | 152 6 | * |
| Lactate (mEq/l) | 2 3 | (6) | 3 0 | (7) | 0 7 | — |

$\bar{x}$ – Mean value The number of determinations are given in parenthesis
* – $p < 0\ 05$

*Pregnant women*

*Diabetic mothers (DM)* The eight diabetic mothers aged 17 to 31 years had had diabetes for at least 10 years and all were treated with insulin Four were primiparous Three of the women (nos 3 5 and 6) had retinopathy before pregnancy During pregnancy

Table 2 *The effects of maternal diabetes on different chemical constituents in umbilical venous plasma*

Comparison between the values of the control and the diabetes study

| Analysis | $\bar{x}_{control}$ | | $\bar{x}_{diabetes}$ | | Difference | Significance |
|---|---|---|---|---|---|---|
| pH | 7 460 | (8) | 7 358 | (8) | 0 102 | * |
| $pCO_2$ (mm Hg) | 29 0 | (8) | 37 0 | (8) | 8 0 | * |
| $BE_p$ (mEq/l) | −3 7 | (8) | −4 5 | (8) | 0 8 | — |
| $K^+$ (mEq/l) | 4 35 | (8) | 3 89 | (8) | 0 46 | — |
| $Na^+$ (mEq/l) | 136 9 | (8) | 134 9 | (8) | 2 0 | — |
| $Cl^-$ (mEq/l) | 110 3 | (8) | 106 1 | (7) | 4 2 | — |
| $HCO_3^-$ (mEq/l) | 19 9 | (8) | 19 8 | (8) | 0 1 | — |
| $Ca^{++}$ (mEq/l) | 6 29 | (8) | 5 74 | (7) | 0 55 | — |
| Total protein (mEq/l) | 12 6 | (8) | 11 8 | (8) | 0 8 | — |
| $HPO_4^{--}$ (mM/l) | 1 84 | (8) | 2 03 | (7) | 0 19 | — |
| Glucose (mg/100 ml) | 83 0 | (7) | 131 5 | (6) | 48 5 | |
| Lactate (mEq/l) | 2 3 | (7) | 2 3 | (7) | 0 0 | — |

$\bar{x}$ – Mean value The number of determinations are given in parenthesis
* – $p < 0\ 05$ ** – $p < 0\ 01$

Table 3 *The effects of maternal diabetes on different chemical constituents in umbilical arterial plasma*

Comparison between the values of the control and the diabetes study

| Analysis | $\bar{x}_{control}$ | | $\bar{x}_{diabetes}$ | | Difference | Significance |
|---|---|---|---|---|---|---|
| pH | 7 384 | (8) | 7 328 | (8) | 0 056 | * |
| $pCO_2$ (mm Hg) | 38 5 | (8) | 43 6 | (8) | 5 1 | * |
| $BE_p$ (mEq/l) | −1 8 | (8) | −2 7 | (8) | 0 9 | — |
| $K^+$ (mEq/l) | 4 58 | (8) | 3 84 | (8) | 0 74 | — |
| $Na^+$ (mEq/l) | 137 0 | (7) | 133 1 | (8) | 3 9 | — |
| $Cl^-$ (mEq/l) | 109 5 | (8) | 106 7 | (7) | 2 8 | — |
| $HCO_3^-$ (mEq/l) | 22 3 | (8) | 21 9 | (8) | 0 4 | — |
| $Ca^{++}$ (mEq/l) | 6 36 | (8) | 5 54 | (7) | 0 82 | — |
| Total protein (mEq/l) | 12 8 | (8) | 12 3 | (8) | 0 5 | — |
| $HPO_4^{--}$ (mM/l) | 1 71 | (8) | 2 09 | (7) | 0 38 | — |
| Glucose (mg/100 ml) | 78 4 | (7) | 70 9 | (5) | 0 6 | — |
| Lactate (mEq/l) | 2 4 | (7) | 3 0 | (7) | 0 6 | — |

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*Healthy mothers (HM)* The eight healthy mothe in the control group were aged 20 to 38 Caesarea section was required for cephalo pelvic disproportio All mothers received a slow infusion of 5 per ce glucose during operation In the diabetic group t infusion was begun half an hour earlier together wi a calculated amount of insulin Thus the diabet mother had received about 25 3 g glucose prior operation which theoretically might raise their plas glucose level by about 100–150 mg/100 ml

*Blood sampling*

Every operation and blood sampling were perform by the same obstetrician After the uterus had bee opened and the infant delivered 0 7 ml blood w taken by puncture from the umbilical artery and ve respectively within / 1 / minutes (umbilical ve x 7/10 minutes umbilical artery x = 9/10 minute and with the placenta *in situ* At the same time sample of blood (0 7 ml) from a peripheral vein the mother was taken without stasis After th samples were drawn they were quickly transferred heparinized microliter test tubes The test tubes we capped mixed and placed in ice water Blood pH w measured at 37 C and within 15 minutes usin Sanz capillary glass electrode and Radiometer P Meter 4 (18) The plasma total CO was determin on a Kopp Natelson microgasometer within an ho (18) The plasma was kept in a deep freeze for ele trolyte analysis Microliter chemical methods we

used for determination of potassium sodium chloride calcium total protein inorganic phosphorus glucose and lactate (17 18) pCO BE and HCO were calculated from blood pH and plasma total CO using Siggaard Andersens alignment nomogram (15)

## RESULTS

The differences in mean values between healthy mothers (HM) and diabetic mothers (DM) and the differences in mean values between newborn infants of healthy mothers (IHM) and newborn infants of diabetic mothers (IDM) were calculated and are presented in Tables 1 2 and 3 In Figs 1 and 2 the mean values ±1 standard deviation are plotted The means of individual differences of the chemical constituents between umbilical vein (UV) and umbilical artery (UA) and between umbilical vein (UV) and maternal vein (MV) were calculated and are presented in Tables 4 and 5

### *pH*

The mean pH was high in both HM and DM The mean umbilical venous pH of IHM was

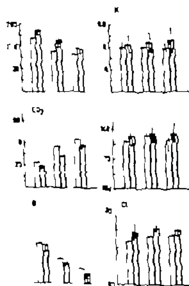


Fig 1 Mean values (± 1 S D) of pH pCO, BE, K Na and Cl in blood of maternal vein (MV) umbilical vein (UV) and umbilical artery (UA) in the diabetic and the control group The hatched columns represent the values of the control group (pCO in mm Hg BE, K Na and Cl in mEq/l)

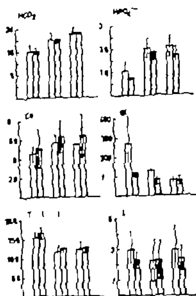


Fig 2 Mean values (±1 S D) of HCO Ca HPO total protein glucose and lactate in blood of maternal vein (MV) umbilical vein (UV) and umbilical artery (UA) in the diabetic and the control group The hatched columns represent the values of the control group (Glucose in mg/100 ml HPO in mM/l plasma electrolytes in mEq/l)

also high The mean pH of DM and IDM tended to be below that of HM and IHM A significant difference was obtained between umbilical venous pH of the two groups

The mean of individual differences in blood pH between maternal vein and umbilical vein of the diabetic group was significant and probably significant in the control group

### *pCO*

The pCO values in maternal venous blood of both groups were rather low There was no significant difference in the pCO values in either maternal or early neonatal blood in the diabetic and the control group

The differences in mean pCO levels between maternal venous and umbilical venous samples in both groups were highly significant In both IDM and IHM the mean umbilical arterial pCO was higher than the umbilical venous pCO

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*Healthy mothers (HM)* The eight healthy mothers in the control group were aged 20 to 38 Caesarean section was required for cephalo pelvic disproportion All mothers received a slow infusion of 5 per cent glucose during operation In the diabetic group the infusion was begun half an hour earlier together with a calculated amount of insulin Thus the diabetic mother had received about 2 5–3 g glucose prior to operation which theoretically might raise their plasma glucose level by about 100–150 mg/100 ml

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|---|---|---|---|---|---|---|
| pH | 7 460 | (8) | 7 358 | (8) | 0 102 | ** |
| $pCO_2$ (mm Hg) | 29 0 | (8) | 37 0 | (8) | 8 0 | |
| $BE_p$ (mEq/l) | −3 7 | (8) | −4 5 | (8) | 0 8 | — |
| K (mEq/l) | 4 35 | (8) | 3 89 | (8) | 0 46 | — |
| $Na^{+}$ (mEq/l) | 136 9 | (8) | 134 9 | (8) | 2 0 | — |
| $Cl^{-}$ (mEq/l) | 110 3 | (8) | 106 1 | (7) | 4 2 | — |
| $HCO^{-}$ (mEq/l) | 19 9 | (8) | 19 8 | (8) | 0 1 | — |
| $Ca^{++}$ (mEq/l) | 6 29 | (8) | 5 74 | (7) | 0 55 | — |
| Total protein (mEq/l) | 12 6 | (8) | 11 8 | (8) | 0 8 | — |
| $HPO_4^{--}$ (mM/l) | 1 84 | (8) | 2 03 | (7) | 0 19 | — |
| Glucose (mg/100 ml) | 83 0 | (7) | 131 5 | (6) | 48 5 | |
| Lactate (mEq/l) | 2 3 | (7) | 2 3 | (7) | 0 0 | — |

$\bar{x}$ – Mean value The number of determinations are given in parenthesis
* – $p < 0 05$ ** – $p < 0 01$

Table 3 *The effects of maternal diabetes on different chemical constituents in umbilical arterial plasma*

Comparison between the values of the control and the diabetes study

| Analysis | $\bar{x}_{control}$ | | $\bar{x}_{diabetes}$ | | Difference | Significance |
|---|---|---|---|---|---|---|
| pH | 7 384 | (8) | 7 328 | (8) | 0 056 | * |
| $pCO_2$ (mm Hg) | 38 5 | (8) | 43 6 | (8) | 5 1 | * |
| $BE_p$ (mEq/l) | −1 8 | (8) | −2 7 | (8) | 0 9 | — |
| $K^{+}$ (mEq/l) | 4 58 | (8) | 3 84 | (8) | 0 74 | — |
| $Na^{+}$ (mEq/l) | 137 0 | (7) | 133 1 | (8) | 3 9 | — |
| Cl (mEq/l) | 109 5 | (8) | 106 7 | (7) | 2 8 | — |
| $HCO_3^{-}$ (mEq/l) | 22 3 | (8) | 21 9 | (8) | 0 4 | — |
| $Ca^{+}$ (mEq/l) | 6 36 | (8) | 5 54 | (7) | 0 82 | — |
| Total protein (mEq/l) | 12 8 | (8) | 12 3 | (8) | 0 5 | — |
| $HPO_4^{--}$ (mM/l) | 1 71 | (8) | 2 09 | (7) | 0 38 | — |
| Glucose (mg/100 ml) | 78 4 | (7) | 70 9 | (5) | 0 6 | — |
| Lactate (mEq/l) | 2 4 | (7) | 3 0 | (7) | 0 6 | — |

$\bar{x}$ – Mean value The number of determinations are given in parenthesis
* – $p < 0 05$

### *Blood sampling*

Every operation and blood sampling were performed by the same obstetrician After the uterus had been opened and the infant delivered 0 7 ml blood was taken by puncture from the umbilical artery and vein respectively within /₄–1 /₄ minutes (umbilical vein – 7/10 minutes umbilical artery – 9/10 minutes) and with the placenta *in situ* At the same time a sample of blood (0 7 ml) from a peripheral vein of the mother was taken without stasis After the samples were drawn they were quickly transferred to *heparinized microliter test tubes* The test tubes were capped mixed and placed in ice water Blood pH was measured at 37 C and within 15 minutes using a Sanz capillary glass electrode and Radiometer pH Meter 4 (18) The plasma total $CO_2$ was determined on a Kopp Natelson microgasometer within an hour (18) The plasma was kept in a deep freeze for electrolyte analysis Microliter chemical methods were

used for determination of potassium, sodium chloride calcium total protein, inorganic phosphorus glucose and lactate (17 18) $pCO_2$, BE, and HCO were calculated from blood pH and plasma total CO using Siggaard Andersens alignment nomogram (15)

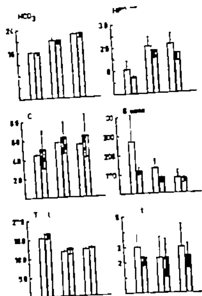


Fig 2 Mean values (±1 S D) of HCO Ca HPO — total protein glucose and lactate in blood of maternal vein (MV) umbilical vein (UV) and umbilical artery (UA) in the diabetic and the control group The hatched columns represent the values of the control group (Glucose in mg/100 ml HPO — in mM/l plasma electrolytes in mEq/l)

## RESULTS

The differences in mean values between healthy mothers (HM) and diabetic mothers (DM) and the differences in mean values between newborn infants of healthy mothers (IHM) and newborn infants of diabetic mothers (IDM) were calculated and are presented in Tables 1 2 and 3 In Figs 1 and 2 the mean values ±1 standard deviation are plotted The means of individual differences of the chemical constituents between umbilical vein (UV) and umbilical artery (UA) and between umbilical vein (UV) and maternal vein (MV) were calculated and are presented in Tables 4 and 5

### *pH*

The mean pH was high in both HM and DM The mean umbilical venous pH of IHM was also high The mean pH of DM and IDM tended to be below that of HM and IHM A significant difference was obtained between umbilical venous pH of the two groups

The mean of individual differences in blood pH between maternal vein and umbilical vein of the diabetic group was significant and probably significant in the control group

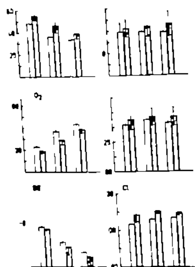


Fig 1 Mean values (± 1 S D) of pH pCO BE, K Na and Cl in blood of maternal vein (MV) umbilical vein (UV) and umbilical artery (UA) in the diabetic and the control group The hatched columns represent the values of the control group (pCO in mm Hg BE K Na and Cl in mEq/l)

### *$pCO_2$*

The pCO values in maternal venous blood of both groups were rather low There was no significant difference in the pCO values in either maternal or early neonatal blood in the diabetic and the control group

The differences in mean pCO levels between maternal venous and umbilical venous samples in both groups were highly significant In both IDM and IHM the mean umbilical arterial pCO was higher than the umbilical venous pCO

Table 4 *The means of individual differences and their significance for chemical constituents in blood plasma of umbilical vein umbilical artery and maternal vein*

Control study

| Analysis | Umbilical vein–umbilical artery | | | Umbilical vein–maternal vein | | |
|---|---|---|---|---|---|---|
| | *n* | Difference | Significance | *n* | Difference | Significance |
| pH | 8 | 0 076 | * | 8 | 0 091 | |
| $pCO_2$ (mm Hg) | 8 | 9 5 | *** | 8 | 10 1 | * * |
| BEp (mEq/l) | 8 | 1 85 | * | 8 | 3 25 | * |
| $K^+$ (mEq/l) | 8 | 0 23 | — | 8 | 0 19 | — |
| Na (mEq/l) | 8 | 0 1 | — | 8 | 1 7 | — |
| Cl (mEq/l) | 8 | 0 8 | — | 8 | 2 0 | — |
| $HCO_3^-$ (mEq/l) | 8 | 2 4 | * | 8 | 3 8 | * * |
| $Ca^{++}$ (mEq/l) | 8 | 0 07 | — | 8 | 1 19 | — |
| Total protein (mEq/l) | 8 | 0 28 | — | 8 | 4 00 | * * |
| $HPO_4^{--}$ (mM/l) | 8 | 0 13 | — | 8 | 1 11 | * * |
| Glucose (mg/100 ml) | 7 | 4 6 | — | 7 | 34 6 | |
| Lactate (mEq/l) | 7 | 0 1 | — | 7 | 0 0 | — |

*n* – Number of determinations
* – $p<0.05$ ** – $p<0.01$ * * – $p<0.001$

### $BE_p$

The mean level of metabolic acidosis was greater (by about 3 to 5 mEq/l) in maternal plasma compared with plasma from the umbilical vessels The increase of metabolic acidosis in maternal blood did not compensate for the reduced maternal pCO levels which is seen in the high maternal pH The early neonatal $BE_p$ values were similar in both groups

The differences in the mean $BE_p$ levels between maternal vein and umbilical vein samples of the control group were highly significant This was not seen in the diabetic group but there was wider individual variations in this group

### *Potassium*

The mean levels of potassium were similar in both early neonatal and maternal blood plasma in both groups

### *Sodium*

No differences were observed between the sodium levels in early neonatal and maternal blood plasma in either group

### *Chloride*

The plasma chloride levels were of the same magnitude in early neonatal and maternal blood in both groups

### *Bicarbonate*

In both groups the concentrations of plasma bicarbonate in maternal vein were significantly lower than those found in the umbilical vessels The mean levels were similar in both the control and the diabetic group The mean bicarbonate concentration increased gradually from maternal and umbilical vein to umbilical artery samples

### *Calcium*

No differences were observed between the calcium levels in early neonatal and maternal

Table 5 *The means of individual differences and their significance for chemical constituents in blood plasma of umbilical vein umbilical artery and maternal vein*

Diabetes study

| Analysis | Umbilical vein–umbilical artery | | | Umbilical vein–maternal vein | | |
|---|---|---|---|---|---|---|
| | *n* | Difference | Significance | *n* | Difference | Significance |
| pH | 8 | 0 030 | — | 8 | 0 121 | * |
| $pCO_2$ (mmHg) | 8 | 6 6 | | 8 | 14 1 | * |
| BEp (mEq/l) | 8 | 1 76 | — | 8 | 2 75 | * |
| K (mEq/l) | 8 | 0 05 | — | 8 | 0 01 | — |
| Na (mEq/l) | 8 | 1 8 | — | 8 | 3 3 | — |
| Cl (mEq/l) | 7 | 0 6 | — | 7 | 2 8 | — |
| HCO (mEq/l) | 8 | 2 0 | — | 8 | 3 6 | * |
| Ca (mEq/l) | 7 | 0 20 | — | 7 | 1 24 | |
| Total protein (mEq/l) | 8 | 0 48 | — | 8 | 3 54 | * |
| $HPO_4^{--}$ (mM/l) | 7 | 0 06 | — | 7 | 0 96 | * |
| Glucose (mg/100 ml) | 6 | 52 5 | — | 6 | 138 7 | |
| Lactate (mEq/l) | 7 | 0 7 | — | 7 | 0 7 | — |

*n* – Number of determinations
* – $p<0.05$ ** – $p<0.01$ – $p<0.001$

blood plasma of the control and the diabetic groups although the early neonatal calcium level tended to be higher than the maternal

*Total protein*

The concentration of total protein in early neonatal plasma was significantly below that of the maternal in all cases No difference was observed between the mean concentrations of the umbilical vessel samples or between the control and diabetic groups

*Inorganic phosphorus*

The concentration of inorganic phosphorus in the umbilical vessels of both groups was similar The increase in inorganic phosphorus in early neonatal compared with maternal plasma was highly significant in both groups

*Glucose*

The plasma glucose of DM and IDM tended to be higher than that of the control group The plasma glucose levels of the umbilical artery samples were similar in both groups

*Lactate*

The lactate levels were similar in both early neonatal and maternal blood in both groups

## DISCUSSION

The blood samples for this study were all obtained during general anaesthesia for caesarean section The general anaesthesia caused a fall in maternal pCO due to hyperventilation This resulted in considerable respiratory alkalosis seen in the maternal blood at operation more than that normally occurring during pregnancy (6 11)

Early neonatal blood was obtained from the umbilical vessels and the maternal blood from a peripheral vein The sampling sites made it possible to assess the acid base and electrolyte status of the infant itself and also to some extent to compare the values between early neonatal and maternal blood

*Maternal blood*

The acid base balance of the diabetic mother did not differ significantly from that of the healthy mother This confirmed that the diabetes was well-controlled In both groups the blood pH was increased due to the fall in pCO associated with hyperventilation The respiratory alkalosis which normally occurs during pregnancy was thus further increased

In four of the diabetic women pH pCO and $BE_p$ were determined in peripheral venous blood on the morning of the day prior to operation The pH ranged from 7 38 to 7 53 ($\bar{x}$ = 7 465) pCO from 25 to 32 mm Hg ($\bar{x}$ = 28 3) $BE_p$ from −2 1 to −6 5 mEq/l ($\bar{x}$ = −3 95) These values are in agreement with those reported by MacRae & Palavradji (6) in capillary blood from pregnant women with well-controlled diabetes

At operation the $pCO_2$ decreased the $BE_p$ decreased leading to a rise of mean pH from 7 465 to 7 479

The residual anion fraction calculated as (Na + $K^+$ + Ca + 2) − (Cl + $HCO_3^-$ + Prot ) mEq/l was small in both HM and DM 5 4 mEq/l and 6 1 mEq/l respectively This indicated that the metabolic acidosis measured as $BE_p$ was not due to an increase of free acids but to a reduction of the bicarbonate concentration as a result of a compensatory mechanism to readjust pH

Most of the plasma electrolytes were similar in HM and DM The level of inorganic phosphorus was somewhat lower in the plasma of HM and the level of glucose higher in the plasma of DM The increased glucose level of DM was due partly to glucose infusion prior to and during operation as well as the underlying disease

In the blood plasma of HM the osmolar activity of glucose was about 6 5 mOsm/l while the osmolar activity of glucose in DM was about 15 mOsm/l The increased osmotic activity in blood plasma of DM attracts water from the cells and results in dilution of the constituents in the extracellular fluid In the present study this is reflected in the slightly

lower concentration of potassium sodium, chloride calcium and total protein in DM

*Newborn infant blood*

The acid base balance of the umbilical arterial blood of IDM and IHM did not differ significantly This observation is in good agreement with the opinion of Prod hom *et al* (10)

Recently it has been stated that newborn infants delivered by caesarean section are likely to have severe acidosis if the maternal pCO level is lowered below 17 mm Hg (7)

In the present study one of the HM (02) and one of the DM (07) had pCO levels below 17 mm Hg but early neonatal acidosis was not recorded in these cases Moya *et al* (7) proposed that intense maternal hyperventilation is associated with a change in uterine and placental circulation with vasoconstriction which causes fetal acidosis The absence of early neonatal acidosis in the infants of the present study suggests that at the time of blood sampling there was adequate feto maternal circulation and acid base relationship

However there was a difference between the acid base status in the umbilical venous blood of the diabetic and the healthy group due to a significantly lower pH in IDM This difference was related to the larger drop in pH between maternal and umbilical venous blood in the diabetic group The slightly higher hydrogen ion concentration in the blood of the IDM and the more pronounced difference between early neonatal and maternal pH could suggest that the IDM in utero existed at a somewhat higher hydrogen ion concentration but that this was not outside normal limits

The mean levels of the plasma electrolytes determined were similar in both IDM and IHM Compared to the maternal levels the early neonatal concentrations of bicarbonate inorganic phosphorus and total protein differed significantly

The difference in maternal and early neonatal bicarbonate concentration was due to the marked reduction of *maternal* plasma bicarbonate Thereby to some extent there was compensation for the decrease of pCO by hyperventilation

The concentration of inorganic phosphorus in early neonatal plasma was about twice that of the maternal This difference between maternal and early neonatal levels of inorganic phosphorus was similar to that found normally at term (18) The concentration of maternal and early neonatal inorganic phosphorus is consistently lower than that reported by Kaiser & Goodlin (4) The higher pH in the present study compared to that of these authors (4) is probably partly responsible for the lower concentration of inorganic phosphorus, as plasma phosphorus is known to vary inversely with pH (15 18)

The early neonatal plasma level of total protein of the present study corresponds very closely to the values reported by Nicolopoulos & Smith (9)

The highly significant difference between early neonatal and maternal concentrations of inorganic phosphorus and total protein suggests active transport of these substances through placenta The higher early neonatal levels of calcium and inorganic phosphorus compared with the maternal levels probably indicates a fetal utilization in utero

The mean concentration of glucose in the umbilical vein was higher in IDM than IHM while the glucose level in the umbilical artery was similar in both groups The arterio venous difference of glucose in the umbilical vessels of IDM was about ten times greater than that of IHM This was due to the high concentration of glucose in the umbilical vein

The excess glucose crossing the placenta to the IDM was not reflected in the blood leaving the infant as the IDM had a similar level of glucose in the umbilical artery as the IHM Stembera & Hodr (16) reported that the arterio venous glucose difference in healthy fetuses became greater the higher the concentration in the umbilical vein Recently it has been shown that concomitantly with a rise in maternal blood sugar there is a rise in fetal blood sugar followed by an increase in fetal plasma

insulin (8) and also that the baby of the diabetic mother has a greater rise in plasma insulin than the baby of a healthy mother in response to a glucose load (1)

Thus the values of the arterio-venous glucose difference of the IDM suggested that the IDM seemed to be able to correct for an excess load of glucose and maintain a normal level in the blood in the umbilical artery

The lactate levels in maternal venous and umbilical arterial blood were similar The failure to observe a feto-maternal concentration gradient of lactate was probably due to a slightly increased maternal lactate production from hyperventilation The levels of lactate suggest that the oxygenation of the mother and consequently the fetus during general anesthesia was good

The acid base and electrolyte status of the IDM was found to be within normal limits This was due to the comparatively unchanged acid base and electrolyte balance of the well controlled diabetic mother The high glucose level of the diabetic mother was however not reflected in the umbilical artery blood of the infant The results presented in this study demonstrate that the acid base and electrolyte status of the newborn infant of a well-controlled diabetic mother delivered by caesarean section does not differ significantly from that of a newborn infant of a healthy mother also delivered by caesarean section


### SUMMARY

The acid base and electrolyte balance was studied in 8 diabetic and 8 healthy mothers and their infants at caesarean section near term

Microliter methods were used for determination of pH total CO potassium sodium chloride calcium total protein inorganic phosphorus glucose and lactate

The acid base and electrolyte balance of the newborn infant of a well-controlled diabetic mother did not differ significantly from that of a newborn infant of a healthy mother


The infant of a diabetic mother seemed to be able to correct for an excess load of glucose and to maintain a normal level in the blood in the umbilical artery

Earlier observations that intense maternal hyperventilation is associated with a changed uterine and placental circulation and results in fetal acidosis were not confirmed in the present study


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# THE DIAGNOSIS OF IRON DEFICIENCY ANEMIA IN CHILDREN

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Although iron deficiency is the most common cause of anemia after the first months of life there still seem to be problems in the establishment of a definite diagnosis of iron deficiency anemia particularly in cases complicated by infection.

Iron deficiency is characterized by hypochromasia microcytosis hyposideremia and hypertransferrinemia But some of these criteria may be lacking and they are not pathognomonic of iron deficiency anemia Normal red blood cells indices are usually found in a mild degree of iron deficiency anemia (8 9) Hypochromasia and microcytosis on the other hand are said to occur in anemia of infection and hemolytic anemias (15 16) Relatively high serum iron concentration has been reported in cases of iron deficiency anemia (3 4 8) and even severe hyposideremia may be seen in infectious disorders (1 6 11)

The purpose of this investigation has been to try to assess the possibility of making a definite diagnosis of iron deficiency anemia on examination of a larger group of children and to study the different parameters in the evaluation of the state of iron deficiency

## MATERIAL AND METHODS

Attempts were made to have all cases of iron deficiency [illegible] admitted to the hospital in a 2 year period of time included in the study Nine of 40 cases of probable iron deficiency (degree of anemia and are defined below) had to be excluded due to lack of transferrin studies. All of them had low MCHC and MCH Whenever sufficient blood was available transferrin studies were also performed on a corresponding number of patients drawn from 51 cases of secondary anemia admitted in the same period of time Sixty two children with hemoglobin concentration below 10 g per 100 ml aged 9 months to 11 years (only three above 8 years of age) were thus selected for studies Twenty nine children with hemoglobin concentration above 11.2 g per 100 ml have been used as a control group

Hemoglobin concentration, hematocrit and red blood cell counts were determined in duplicate by methods reported previously (8 10)

The serum iron (SI) was determined by the Central Laboratory at this hospital by the method of Ramsay (13) total iron binding capacity (TIBC) by the Central Laboratory Ullevål Sykehus Oslo by the method of Ramsay (14)

## RESULTS

The hemoglobin concentration in the 62 anemic children ranged from 3 2 to 9 9 g per 100 ml

A diagnosis of iron deficiency anemia was made in 31 cases (Table 1) The diagnosis was based on (clinical history) low cell indices changes in peripheral blood smears hyposideremia, hypertransferrinemia and low saturation index Therapeutic response to iron therapy was demonstrated in all the 26 cases (Table 1) which it was possible to follow up including two cases with absorption deficiency which responded to parenteral iron therapy Infection was present in nearly [illegible] of the cases of iron deficiency anemia

The remaining 31 anemic cases comprise a

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Table 2 *Distribution of red cell indices in the three groups of children*

| Group | MCHC <28 | Per cent 28–28.9 | 29–29.9 | >30 | No of cases |
|---|---|---|---|---|---|
| Iron deficiency anemia | 23 | 6 | 2 | 0 | 31 |
| Secondary anemias | 1 | 0 | 3 | 25 | 29 |
| Control | 0 | 0 | 3 | 26 | 29 |

| Group | MCH <22 | pg 22–23.9 | 24–24.9 | >25 | No of cases |
|---|---|---|---|---|---|
| Iron deficiency anemia | 28 | 3 | 0 | 0 | 31 |
| Secondary anemias | 1 | 4 | 4 | 22 | 31 |
| Control | 0 | 2 | 3 | 24 | 29 |

| Group | MCV <70 | μ³ 70–74.9 | 75–79.9 | >80 | No of cases |
|---|---|---|---|---|---|
| Iron deficiency anemia | 14 | 8 | 7 | 2 | 31 |
| Secondary anemias | 0 | 4 | 2 | 23 | 29 |
| Control | 0 | 1 | 3 | 25 | 29 |

values were repeatedly seen in several hematologically normal children. Low SI values were also found in some cases of secondary anemia.

Fig. 2 shows the distribution of TIBC in the three groups of children. Values above 400 μg per 100 ml were seen in all cases of iron deficiency except a temporary low value (a later high value used in the figure) in a child with osteomyelitis. Values above 400 μg per 100 ml occurred in four of the hematologically normal children but only in one (414 μg per 100 ml) of the 31 cases of secondary anemia, a child with leucemia.

Fig. 3 shows the distribution of transferrin saturation. Low values were seen in all cases of iron deficiency anemia. Low values were however also found in several cases in the control group and six children with secondary anemia had a saturation index of 8 to 16 per cent.

## DISCUSSION

The diagnosis iron deficiency anemia should be obvious in the 31 cases presented in Table 1. Although the possibility of iron deficiency

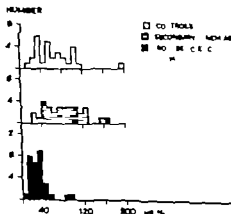


Fig. 1. Distribution of serum iron in the three groups of children.

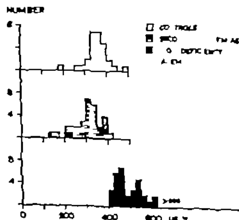


Fig. 2. Distribution of TIBC in the three groups of children.

Table 1 *Iron deficiency anemia*

| No | Before treatment<br>Age | Hgb (g/100 ml) | MCHC ( ) | MCH (μg ) | MCV ($\mu^3$) | SI (μg ) | TIBC (μg ) | Saturation ( ) | During treatment Hgb (g/100 ml) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 9 mths | 3 2 | 25 | 17 | 69 | 95 | 711 | 13 4 | 11 6 |
| 2 | 3 yrs | 3 7 | 23 | 14 | 62 | 85 | 584 | 14 8 | 9 8 |
| 3 | 13 mths | 4 7 | 22 | 13 | 59 | 50 | 462 | 10 8 | 11 7 |
| 4 | 16 mths | 5 4 | 27 | 16 | 61 | 13 | 411 | 3 1 | 11 9 |
| 5 | 7 yrs | 5 8 | 21 | 13 | 63 | 37 | 766 | 4 8 | |
| 6 | 6 yrs | 6 8 | 23 | 19 | 84 | 13 | 465 | 2 8 | 11 2 |
| 7 | 14 mths | 7 5 | 28 | 19 | 70 | 20 | 522 | 3 8 | 11 5 |
| 8 | 11 yrs | 7 5 | 27 | 15 | 56 | 26 | 471 | 5 5 | 11 0 |
| 9 | 8 yrs | 7 5 | 25 | 16 | 64 | 24 | 506 | 4 9 | 11 9 |
| 10 | 17 mths | 7 6 | 26 | 19 | 73 | 23 | 432 | 5 3 | 11 2 |
| 11 | 4 yrs | 7 6 | 27 | 21 | 78 | 40 | 438 | 9 1 | 10 5 |
| 12 | 1 yr | 8 1 | 27 | 16 | 59 | 33 | 438 | 7 6 | 11 2 |
| 13 | 7 yrs | 8 1 | 28 | 23 | 80 | 17 | 506 | 3 3 | 11 9 |
| 14 | 3 yrs | 8 1 | 28 | 18 | 65 | 22 | 438 | 5 0 | |
| 15 | 2 yrs | 8 2 | 26 | 20 | 77 | 21 | 432 | 4 8 | 11 3 |
| 16 | 8 yrs | 8 3 | 27 | 17 | 65 | 32 | 538 | 5 9 | 10 1 |
| 17 | 9 yrs | 8 4 | 28 | 20 | 70 | 47 | 534 | 8 8 | 10 5 |
| 18 | 1 yr | 8 4 | 26 | 17 | 66 | 56 | 548 | 10 3 | 12 0 |
| 19 | 1½ yrs | 8 7 | 29 | 20 | 68 | 31 | 466 | 6 6 | 11 6 |
| 20 | 2 yrs | 8 7 | 26 | 20 | 78 | 31 | 476 | 6 5 | 10 9 |
| 21 | 9 yrs | 9 2 | 27 | 20 | 75 | 18 | 410 | 4 4 | 10 1 |
| 22 | 5 yrs | 9 2 | 27 | 20 | 75 | 9 | 465 | 1 9 | 11 3 |
| 23 | 1 yr | 9 3 | 26 | 16 | 62 | 17 | 465 | 3 6 | |
| 24 | 1½ yrs | 9 3 | 27 | 20 | 74 | 16 | 534 | 2 9 | |
| 25 | 9 mths | 9 5 | 27 | 19 | 71 | 15 | 492 | 3 0 | 12 5 |
| 26 | 10 mths | 9 7 | 27 | 19 | 70 | 50 | 410 | 11 7 | 11 5 |
| 27 | 1 yr | 9 7 | 27 | 20 | 74 | 35 | 574 | 6 1 | 11 3 |
| 28 | 1 yr | 9 8 | 28 | 18 | 64 | 35 | 574 | 6 1 | 11 3 |
| 29 | 14 mths | 9 8 | 28 | 22 | 77 | 23 | 431 | 5 3 | 11 3 |
| 30 | 1 yr | 9 9 | 29 | 22 | 75 | 23 | 534 | 4 3 | |
| 31 | 18 mths | 9 9 | 27 | 19 | 72 | 33 | 560 | 5 9 | 11 6 |

group with different causes of anemia such as infection malignancy and hemolysis Three cases of acute hemorrhage have also been included all three had normal cell indices SI and TIBC

Table 2 shows the distribution of red cell indices in the three groups of children All but two of the cases with iron deficiency anemia had MCHC below 29 per cent while a value below 29 was only demonstrated in one of the remaining 59 children a case of Hodgkin s disease (also low MCH MCV and SI) Borderline values (29–29 9 per cent) was demonstrated in three cases of iron deficiency anemia and 6 of the other children (Two cases of leucemia and only one case with anemia of infection ) Borderline values of MCH (22–23 9 μg per 100 ml) was demonstrated in three cases of iron deficiency anemia and 6 of the other children

Table 2 shows that although MCV is low in most cases of iron deficiency anemia there is considerable overlapping between the three groups

Changes typical of iron deficiency anemia was only seen in about half of the examined blood smears from the cases of iron deficiency anemia

The SI was low in most of the cases of iron deficiency anemia but values above 40 μg per cent per 100 ml were observed in 6 cases (Fig 1) The two highest values may however have been due to exogenous iron Case 1 had received a small blood transfusion 2 days before blood sample was withdrawn and Case 2 iron therapy irregularly [illegible] Low

seen in infectious disorders Hagberg (4) has shown that acute infection will decrease SI before any changes in TIBC occurs resulting in a decrease in saturation (6 to 21 per cent in 13 cases of infection) SI values below 10 μg per cent may be seen in severe types of infection (1 11) resulting in a per cent saturation below 10

High TIBC and decreased saturation never occur in pure anemia of infection and seem to be highly significant in anemic cases

In this study including only cases with hemoglobin concentration below 10 g per 100 ml hypochromasia was found in almost all cases of iron deficiency anemia and in none of the other two groups except for the child with Hodgkin's disease It seems therefore adequate to use the red cell indices in the diagnosis of iron deficiency anemia in clinical practice For research however it is evident that even definite hypochromasia and microcytosis is not sufficient in the diagnosis of iron deficiency anemia Repeated low red cell indices hyposideremia and severe hypotransferrinemia with no effect of prolonged iron therapy were demonstrated in the child with Hodgkin's disease The same type of finding may be made in primary as well as in other types of secondary hypotransferrinemia Other rare causes of hypochromasia and microcytosis may also exist (7 15 16)

*The final* proof of the existence of iron deficiency anemia is the response to iron therapy The interpretation may however be difficult because of the simultaneous cure of *infection* Correction of iron deficiency anemia however usually takes more time

## SUMMARY AND CONCLUSION

Sixty two anemic and 29 hematologically normal children have been studied A diagnosis of iron deficiency anemia was made in 31 cases On the basis of the results it may be stated that it should be possible to establish a definite diagnosis of iron deficiency anemia in a larger group of children with hemoglobin concentration below 10 g per 100 ml even in cases complicated by ordinary types of infections All the parameters in the determination of iron deficiency should be used in scientific work, and control groups (hematologically normal and cases of secondary anemia) ought to be included

In anemic cases elevated TIBC seems to be the most reliable criterion of iron deficiency anemia Hyposideremia is almost always present but is a frequent finding in infectious disorders malignancy and normal children Low transferrin saturation may be seen in infectious disorders as well as in children with normal hematological status However low transferrin saturation in anemic cases *with* elevated TIBC seems to be highly significant

In clinical practice the use of red cell indices seems to be *adequate in the diagnosis* of iron deficiency anemia provided normal values have been established A therapeutic trial will usually confirm the diagnosis of iron deficiency although the possibility of normalization of the hemoglobin *concentration by the* simultaneous *cure of an infection* must also be born in mind

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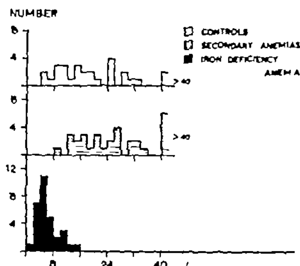



*Fig 3* Distribution of transferrin saturation in the three groups of children

anemia in some of the cases in the control group and the group of secondary anemia cannot be ruled out, it has not been possible either by diagnostic studies or by following up some of the children to disclose iron deficiency in any of the 60 children

The most difficult differentiation is between iron deficiency and anemia of infection Infections are often seen in cases of iron deficiency anemia However this study clearly demonstrates that it is usually possible to demonstrate iron deficiency in spite of complicating infection

*Infection tends to lower the TIBC and it is* a general consensus of opinion that low TIBC is always seen in anemia of infection It is *nevertheless evident from this study that ele*vated TIBC usually exists in cases of iron deficiency anemia in spite of the more ordinary types of infection such as otitis bronchitis and pneumonia Several types of infection such as the reported case of osteomyelitis may however result in *low TIBC in spite of the exist*ence of a simultaneous iron deficiency anemia In such cases it should usually be possible to *demonstrate elevated TIBC after the infection* has been eradicated Hagberg (4) also demonstrated elevated TIBC in 16 cases of iron deficiency *anemia Kasper et al* (5) on the other hand, found elevated TIBC in only eight of 15 older patients with iron deficiency anemia In other studies there seems to be a varying number of cases with normal and even *low TIBC included in materials* of iron deficiency anemia (2, 3 12) This may be due to the inclusion of cases of pure secondary anemia, or *low TIBC* caused by complicating severe infection

The use of TIBC in the diagnosis of iron deficiency anemia in this study as well as in Hagberg s study (4) is complicated by the fact that elevated TIBC may be seen in children with normal hematological status Elevated TIBC has, however, not been demonstrated in any other types of anemia but iron deficiency anemia Pathological conditions which impair the synthesis of plasma protein like infectious, hemolytic and malignant disorders, are associated with decreased TIBC (Almost no overlapping exists in Figure 2 between the secondary and iron deficiency groups)

From this study as well as other studies including a control group, it is evident that low SI levels may often be seen in children with a complete normal hematological picture, and there exists considerable overlapping between normal and iron deficiency cases (4 5 8) An even more important fact is that *low SI levels* are also seen in anemia of infection and malignancy (page 141 Fig 1) The most likely explanation of a relatively high SI value in a markedly iron deficient child is exogenous iron This source of error will probably always exist as one hesitates to delay iron studies particularly in severe cases of anemia

Decreased transferrin saturation is usually considered the most significant sign of iron deficiency anemia This is amazing in consideration of the fact that transferrin saturation is *partly dependant on the serum iron level* (which is an unreliable criterion of iron deficiency anemia—see above) A low level of *transferrin saturation was observed in several* hematologically normal children for instance in a child with hemoglobin concentration of 12 3 g per 100 ml and hematocrit of 40 vol per cent More important however is the observation that decreased saturation may be

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# GASTROENTERITIS WITH SECONDARY DISACCHARIDE INTOLERANCE

*An Outbreak in a Premature Unit*

J LLOYD STILL

*From Queen Charlotte's Maternity Hospital London England*

Gastroenteritis may be complicated by secondary disaccharide intolerance leading to further problems in management An epidemic of gastroenteritis occurring in the Premature Unit at Queen Charlotte's Hospital is reported

## THE OUTBREAK

The time sequence and duration of the gastroenteritis (Fig 1) shows that all 5 infants were nursed in the same Unit cases 4 and 5 became ill a few days after discharge and the former was readmitted to another hospital The shortest incubation period occurred in case 3 in whom diarrhoea commenced on the fourth day of life The duration of the acute illness was similar in all cases lasting from 10–13 days

Stool cultures from the infants and staff and culture of the feeds in the Milk Bank were all negative blood and viral cultures were also unrewarding. The aetiology of the gastroenteritis remains uncertain nine other infants in close proximity were unaffected However several infants with a similar illness were seen in widely separated hospitals in the London area at the same time (personal communication)

## CLINICAL FEATURES

Weight loss was always marked and Fig 2 shows the findings in case 3 who lost over [illegible]0% of his body weight The biochemical findings were consistent with the loss of fluid and electrolytes in the stools, all infants developing a severe metabolic acidosis (Tabl 1) hypokalaemia was a further complication in : infants Intravenous fluids were always required be correction of the acidosis was often difficult All : infants were treated with a 5 day course of intramu cular Ampicillin and Cloxacillin at the onset c symptoms

No particular feed was common to all the infants some being on expressed breast milk and others c artificial feeds In none of the infants did the diar rhoea improve until disaccharides were exclude from the feeds Sugar intolerance was suggested b the clinical features of a hungry infant with explo sive watery diarrhoea who became progressively de hydrated Large quantities of reducing substance were detected in the stools and urine by the "Clin test" method and lactose and sucrose were confirmed by chromatography

## RECOVERY PHASE

The extent of recovery from the disaccharide intolerance was assessed by lactose and sucrose load tests which were performed 2–6 weeks after the onset of gastroenteritis By this time the condition of the infants warranted further investigation and the diarrhoea had ceased The tests were performed as follows

A blood sugar was estimated after 4 hours fasting and the infant was weighed A known dose (10 g/kg) of the disaccharide and a carmine marker were then administered and the blood sugars were obtained every 15 minutes for the next 2 hours The urine and stools passed after the load test were examined for sugars by chromatography The infants were weighed again at the end of the test The stool transit time was also noted from the passage of the carmine marker

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charide intolerance has important applications in dietary management.

Gastroenteritis is especially hazardous in premature infants owing to their greater susceptibility to infection and poor renal function. Moreover premature infants have higher death rates from gastroenteritis than full term newborns (6). Seven of the 12 infants with refractory diarrhoea and secondary disaccharide intolerance described by Burke *et al* (2) were premature. Since then they have encountered a further 12 premature infants all with similar clinical features during two epidemics of diarrhoea in a maternity hospital. All 5 infants in our epidemic were premature and Fig. 1 shows that they had been nursed in the same Unit.

In Burke *et al*'s series clinical response and cessation of diarrhoea were always apparent within 24–72 hours of commencing the lactose free milk Nutramigen. However Nutramigen contains the disaccharide sucrose and all our infants were intolerant of sucrose as well as lactose. They were therefore put on the low lactose (less than 0.1 ) milk Galactomin in which the carbohydrate is contained in the form of the monosaccharide glucose. In spite of this milk several of the infants continued to have diarrhoea for periods up to 10 days suggesting either that our infants had a more severe illness than in the Australian series or that the recovery was not necessarily related to the Galactomin. Disaccharide free vitamin preparations were administered and the stools did not contain any disaccharides after the commencement of Galactomin. Townley (10) stated that if intolerance of more than one disaccharide is present, a milk such as Galactomin or a tailor made mixture of protein fats electrolytes and glucose can be employed.

Anderson *et al* (1) found a low stool pH to be characteristic of disaccharide intolerance. However the stool pHs in our 5 infants were not always below 6.5. The stool transit time was often only 1 1/ hours and the combination of intestinal hurry and reduction of enteric ... ibiotics may have been contributary (8 5). Another feature of the illness was the severe metabolic acidosis which was not corrected by 1/6 molar lactate. The replacement fluid of choice in these circumstances is sodium bicarbonate (7) as these infants frequently have high serum lactate levels.

The recovery of lactase activity may be assessed by biopsy of the small intestine (2) but was considered dangerous in our premature infants. A trial of lactose containing milk could have lead to severe relapse and was not used. Jarrett & Holman (4) performed lactose and sucrose load tests (1.75 g/kg) on premature infants at 14 days of age. They concluded that as measured by these load tests lactase and sucrase activities are normal at that age.

Although our load tests were helpful in dietary management, the dose used (10 g/kg) was unnecessarily high. Since this outbreak 2 further newborn infants have been encountered with a similar illness and *E. coli* 0126 and 086 have been isolated respectively. Both infants required a lactose free diet and in the latter case a lactose load test (2 g/kg) performed 5 weeks after the onset of the illness showed a flat response with a recurrence of diarrhoea as in case 1. When repeated 2 weeks later the lactose load (2 g/kg) was tolerated well, there was a normal rise in blood glucose following the test and a lactose containing milk was then substituted with no ill effects. These findings are further evidence that a variety of causative organisms may be responsible for the similar clinical syndrome of gastroenteritis with secondary disaccharide intolerance.

## SUMMARY

Secondary disaccharide intolerance was a feature of the gastroenteritis in all cases and lasted from 2–10 weeks necessitating treatment with a disaccharide free milk. No bacterial or viral pathogens were isolated from the 5 affected infants and all infants in whom disaccharide intolerance was recognised survived. Lactose tolerance tests in the recovery phase were helpful in deciding when to reintroduce a normal (lactose containing) milk.

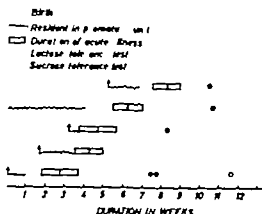



*Fig 1* Time sequence and duration of the epidemic

blood glucose following the sucrose load compared with the lactose load

In case 1 the initial lactose load test (Fig 3 case 1 *a*) was performed 6 weeks after the onset of his illness and provoked a recurrence of his diarrhoea with at weight loss of 105 g following the test The stools and urine were shown to contain large quantities of lactose on the chromatogram As a result of these findings he was maintained on Galactomin until he was 12 weeks old A second lactose tolerance test (Fig 3 case 1 *b*) then showed a satisfactory rise in blood glucose and diarrhoea did not recur

## DISCUSSION

Improvements in Public Health measures knowledge of fluid and electrolyte imbalance, and the advent of antibiotics have all contributed to reduce the infant mortality from gastro-

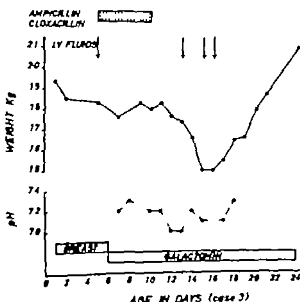



*Fig 2* Changes in weight and capillary pH in case 3

[1] Trufood Formula 17

Table 1 *Changes in weight and severity of the acidosis in the 5 infants*

| Case | Weight (g) Birth | Onset of illness | Lowest | pH | Acidosis Bicarbonate mEq per litre |
|---|---|---|---|---|---|
| 1 | 2100 | 1980 | 1650 | 7 1 | 6 5 |
| 2 | 1520 | 1360 | 1300 | 7 0 | 7 0 |
| 3 | 1930 | 1820 | 1500 | 7 0 | 9 8 |
| 4 | 2050 | 2820 | 2380 | 7 0 | 8 0 |
| 5 | 2220 | 2320 | 2100 | 7 0 | 10 4 |

enteritis in this country Darrow (3) stated that in his experience, nutritional failure had become the most common cause of a fatal termination in infants with diarrhoea In these circumstances antibiotics may be ineffective or even aggravate the condition from resultant superinfection Many of these infants with refractory diarrhoea have now been shown to have secondary disaccharidase deficiencies (9 2) and the recognition of secondary disac

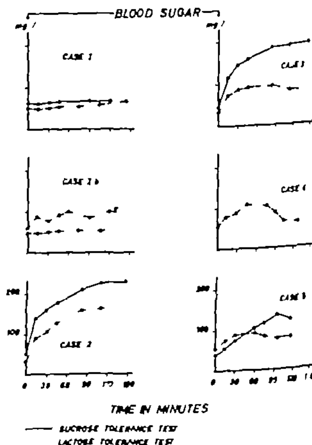



*Fig 3* Oral disaccharide load tests (10g/kg) showing flat response in case Ia and improvement 4 weeks later-case Ib

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# FAMILIAL APLASTIC ANAEMIA WITHOUT CONGENITAL MALFORMATIONS

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Some 40 years ago Fanconi (4) described a family in which hypoplastic anaemia combined with multiple congenital anomalies occurred in 3 brothers Anaemia of the Fanconi type has for many years been the only widely recognized prototype of a constitutional genetically determined aplastic anaemia. The presence of congenital malformations is an essential feature of Fanconi's anaemia It also serves as a useful marker which enables the diagnosis to be made in sporadic cases The recent finding of chromosomal aberrations in Fanconi's anaemia (1 10 11 12 13) further helps to characterize this entity and to establish its genetic nature

Although the incidence of Fanconi's anaemia is probably much higher than can be judged from the number of published cases it comprises a relatively small proportion of cases of aplastic anaemia in childhood Cases not fulfilling the diagnostic criteria for Fanconi's anaemia are usually considered to be acquired even though a certain proportion of such cases remains unaccounted for by any known environmental factor

We have observed a family with 3 siblings all of whom had aplastic anaemia without congenital malformations Furthermore chromosomal changes similar to those found in Fanconi's anaemia were present in these patients This family is presented to illustrate the fact that hereditary aplastic anaemia is not necessarily associated with other congenital malformations In addition our findings suggest that cytogenetic studies are a useful diagnostic tool with which a genetic background may be established in further cases of aplastic anaemia without familial incidence

## CASE REPORTS

Family history The parents are not related The father is healthy The mother suffers from ankylosing spondylitis, for which she received x ray therapy to the neck at the age of 21 years 4 years before she gave birth to E. E. The oldest child a girl E. E., was born in 1955 (case 1) O. E., a boy was born in 1960 (case 2) and T. E., a girl, was born in 1964 (case 3) There are no other children in the family

*Case 1*

E. E., a girl born in 1955 was the first child in the family She was born at term and weighed 2000 g at birth. At the age of 4 / years pancytopenia was first diagnosed. There was no history of an infection or ingestion of drugs preceding the onset of pancytopenia Bone marrow cellularity at that time was judged to be normal. On admission six months later marked pallor and numerous subcutaneous haemorrhages were observed Height and weight were normal for the patient's age The tip of the spleen was just palpable below the costal margin. There were no other abnormal physical findings Laboratory investigations The haemoglobin concentration was 7.3 per 100 ml. the red cell count 2 2 million per mm the haematocrit 21 per cent, the reticulocyte count 0 6 per cent, white blood count 2250 and the platelet count 50 000 per mm Examination of the peripheral blood smear revealed macrocytosis The MCV varied between 103 and 120 cu μ The serum iron ranged between 14.. and 176 μg per 100 ml and the

## ACKNOWLEDGEMENTS

Viral cultures were performed by the Public Health Laboratory Colindale

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*Key words* Secondary disaccharide intolerance infective enteritis prematurity

*Cytogenic studies*

Cytogenetic studies were performed on blood lymphocyte cultures of O E and T E and their mother In the children chromosomal aberrations mainly of the chromatid type were found in a high percentage of mitoses In addition numerous endoreduplicated and tetraploid cells were seen In the mother the changes were of a milder nature Although chromatid breaks were present in some 20 per cent of mitoses usually not more than one affected chromosome per cell was found (Table 2 and Fig 1)

Short term bone marrow cultures[1] were attempted in O E and T E In O E only one cell was available for analysis It showed one dicentric chromosome In T E five cells were analysed and showed chromatid abnormalities

Table 1 *Haematological data in familial aplastic anaemia*

| | E.E. | O E. | T E |
|---|---|---|---|
| Haemoglobin (g/100 ml) | 5 8 | 9 7 | 10 0 |
| Red blood count (million/per mm³) | 1 95 | 2 58 | 3 15 |
| Reticulocyte count ( ) | 0 6 | 1 3 | 0 9 |
| White blood count (per mm³) | 2,250 | 2 800 | 5 750 |
| Platelet count per (mm³) | 14 000 | 25 000 | 30-60 000 |
| Serum iron (μg per 100 ml) | 142 176 | 92 151 | 139 |
| Iron binding capacity (μg per 100 ml) | 242 | 355 | 283 |
| Whole blood folic acid (m μg/ml) | 384 | 199 | 422 |
| Bilirubin | Normal | Normal | Normal |
| Mean corpuscular (volume cu μ) | 103–120 | 107–121 | 100-107 |
| Foetal Hb ( ) | — | 9 0 10 0 | 8 0 |
| ⁵⁹Fe T/2 plasma disappearance (min) | 144 | 144 | — |

## DISCUSSION

The association in Fanconi's anaemia of marrow hypoplasia with a bizarre assortment of congenital malformations affecting a variety of tissues and systems is still a riddle The number and severity of malformations found in individual cases vary considerably Indeed families have been reported in which one member had aplastic anaemia alone while others exhibited the full picture of Fanconi's anaemia with malformations (5 9)

Our family is remarkable for the occurrence of aplastic anaemia in all 3 siblings in the absence of any demonstrable congenital malformations Familial aplastic anaemia without congenital malformations was described by Estren & Dameshek (3) These authors presented 2 such families of French Canadian origin with 3 and 5 affected siblings respectively Cytogenetic techniques were not available at that time Two additional families in which 2 siblings had aplastic anaemia without congenital malformations were described one by Rohr (8) and the other by Nilsson (7) Again no cytogenetic data are available for these families

[1] We thank Dr B Padeh of the Cytogenetic Laboratory of Tel Hashomer Hospital for performing this examination

Table 2 *Incidence and types of chromosome aberrations found in familial aplastic anaemia*

| | Total no of cells analysed | Normal cells (%) | Injured cells (%) | Percentage of cells with: Chromatid break (%) | Chromatid gap (%) | Chromatid exchange ( ) | Endoreduplication (%) | Tetraploid cells ( ) |
|---|---|---|---|---|---|---|---|---|
| O E. | 170 | 22 | 78 | 37 | 8 | 8 | 17 | 10 |
| T E. | 76 | 23 | 76 | 50 | 8 | 5 | 10 | 3 |
| U M | 40 | 45 | 55 | 44 | 10 | 1 6 | | |
| Y E. (mother) | 100 | 77 | 23 | 17 | 2 | | 2 | 1 |

U M is a typical case of Fanconi's anaemia with congenital anomalies but no familial history He is included in the table for comparison.

iron binding capacity was 242 $\mu g$ per 100 ml The bilirubin was 0 3 mg per 100 ml Bone marrow aspiration on admission showed a diminished number of megakaryocytes The cellularity of the myeloid and erythroid series was normal However aspiration done 6 months later revealed a hypocellular marrow with marked erythroid and myeloid hypoplasia and an increase in the number of reticulum cells Ferrokinetic studies showed a prolonged disappearance of $^{59}$Fe from the plasma with a disappearance half time of 144 minutes $^{59}$Fe incorporation into red cells was not determined because of a drop in haemoglobin which necessitated a blood transfusion X ray studies of the skeleton did not show any abnormality and the bone age corresponded to the chronological age The diagnosis of aplastic anaemia cause unknown was made

Prednisone therapy was given for 3 months without any improvement of the pancytopenia The child required numerous blood transfusions Splenectomy which was performed 9 months after the first admission had no beneficial effect Three months later therapy with methyl testosterone 30 mg daily and prednisone 10 mg daily was initiated Six weeks after the beginning of treatment there was a reticulocytosis followed by a rise in haemoglobin up to 12 6 g per 100 ml The white count and platelet count remained unchanged Therapy was stopped after 4 months whereupon a relapse occurred A second course of methyl testosterone and prednisone was initiated followed by a reticulocyte rise up to 7 0 per cent However at that stage the patient developed pyocyaneus sepsis and died No urinary tract anomalies or any other malformations were found on post mortem examination

*Case 2*

O E the younger brother of case 1 was referred to the Haematology Clinic at the age of 4 1/4 years for investigation of a mild anaemia He was born at term His birth weight was 2450 g and his length 45 cm Physical examination revealed a short slender boy His height and weight were below the third percentile for his age His intelligence was good (I Q 120) Patchy hyperpigmented areas were noted over his trunk The spleen and liver were not palpable Examination was otherwise negative Laboratory investigations The haemoglobin concentration was 10 0 per 100 ml red cells 4 150 000 per mm the white blood cell and platelet counts were normal The serum iron was 122 and 151 $\mu g$ per 100 ml on two occasions and the iron binding capacity was 355 $\mu g$ per 100 ml Whole blood folic acid was 199 m$\mu$g/ml Bilirubin glucose 6 phosphate dehydrogenase pyruvic kinase PBI and immunoelectrophoresis of serum proteins were within normal limits Haptoglobin was 34 0 mg per 100 ml The peripheral smear showed macrocytosis a finding which was confirmed by a Price Jones curve The MCV varied between 107 and 120 cu $\mu$ Haemoglobin A was 1 5 per cent and haemoglobin F was 9 and 10 per cent on two occasions A peripheral blood film stained by the acid elution technique showed a small population of red cells containing foetal haemoglobin Haemoglobin $A_2$ and F in the parents were normal The patient was followed in the Haematology Clinic and his condition remained unchanged

At the age of 7 years a few subcutaneous haemorrhages were noted over the lower extremities The platelet count had dropped to 55 000 per mm$^3$ and the white count to 3550 per mm$^3$ A bone marrow aspirate showed normal cellularity with increased deposition of fat Erythropoiesis was increased (M/E ratio 2 5 1) and some macroblasts were seen Megakaryocytes were sparse There was a marked increase in lymphocytes and reticulum cells Skeletal X rays as well as intravenous pyelography were normal Bone age corresponded to the chronological age The half time disappearance of $^{59}$Fe from the plasma was 144 minutes and the red cell uptake of $^{59}$Fe was 82 per cent in 10 days Surface counting over the sacrum remained low while there was an increase in counts over the liver and spleen The plasma iron turnover rate was 0 84 mg/24 hours (normal 1 1 mg/24 hours)

At present at the age of 8 years pancytopenia persists The haemoglobin is 9 7 g per 100 ml the haematocrit 28 per cent reticulocytes 1 3 per cent and the white blood count is 2800 per mm$^3$ with 38 per cent neutrophils and 48 per cent lymphocytes The platelet count is 25 000 per mm

*Case 3*

T E the youngest sister of cases 1 and 2 was referred to our Haematology Clinic at the age of 3½ years because of a mild anaemia She was born at term and her weight at birth was 2250 g Her psychological and motor development were normal Her weight corresponded to the 25th percentile and her length to the 10th percentile for her age Physical examination was unremarkable except for patchy hyperpigmented areas over the body and a few subcutaneous haemorrhages over the lower extremities Laboratory investigations The haemoglobin was 11 7 g per 100 ml red cells 3 440 000 per cu mm the reticulocyte count 0 3 per cent the white blood count 7500 per mm and the platelet count 107 000 per mm A slight macrocytosis was noted in the peripheral blood film and in the Price Jones curve The MCV varied between 100–107 cu $\mu$ Bone marrow aspiration revealed normal cellularity in the myeloid and erythroid series Megakaryocytes were sparse Serum iron was 139 $\mu g$ per 100 ml and the iron binding capacity 283 $\mu g$ per 100 ml Glucose 6 phosphate dehydrogenase pyruvic kinase and bilirubin were within normal limits Haemoglobin A was 2 0 per cent and haemoglobin F 8 0 per cent Skeletal survey and intravenous pyelography were normal Bone age corresponded to the chronological age In the course of the following 6 months the platelet count dropped gradually and is now in the range of 30 000 to 60 000 per mm The haemoglobin concentration is around 10 0 g per 100 ml and the white count is normal

Haematological data relating to the three cases are summarized in Table 1

a reported as a constant finding in Fan is anaemia (1 6 10 11 12 13) In the es of Bloom and his colleagues (1) 10 out 12 patients showed these abnormalities It noteworthy that these authors did not find y chromosomal abnormalities in another up of patients which they classified as nstitutional aplastic anaemia These pa nts showed amegakaryocytic thrombocyto nia soon after birth followed later by pan topenia but did not have congenital anoma s

Chromosomal aberrations most commonly und in lymphocyte cultures from patients th Fanconi s anaemia are chromatid breaks d gaps Breaks may lead to chromatid ex ange dicentric or triradial chromosomes he incidence of mitoses with chromatid aber tions in the material of Bloom and co- orkers (1) ranged between 5 and 45 per cent chroeder *et al* (12) and Schmid *et al* (10) ound a higher incidence between 39 and 67 ercent Endoreduplication is less common in anconi s anaemia *In our patients 53 and 63* er cent of mitoses showed chromatid abnor malities *(Table 2)* Endoreduplication was ound in 17 and 10 per cent of mitoses Tetra ploid mitoses were also common (Fig 1)

The finding of some chromosomal breaks in he mother is of interest The incidence of chromatid breaks in lymphocyte cultures from normal subjects reported from different labora tories varies considerably The number of such breaks found in the mother (Table 2) is prob ably just above the upper limit of normal and may be attributed to the X ray therapy which she received 16 years previously for ankylos ing spondylitis (2)

Do our and similar cases represent an in complete form of Fanconi s anaemia or are they to be regarded as a separate entity? The resemblance of our cases to typical cases of Fanconi s anaemia including the chromosomal abnormalities has already been pointed out This would favour the first alternative On the other hand since the presence of congenital malformations has been an essential feature of Fanconi s anaemia it would seem convenient on clinical grounds to subdivide constitutional hereditary aplastic anaemia into two sub- groups one with congenital malformations (Fanconi type) and the other without Congeni- tal malformations Better knowledge of the mechanism linking congenital malformations with marrow aplasia in Fanconi s anaemia will in the future prove whether such a subdivi sion is justified

*Fanconi s anaemia* is probably transmitted as an autosomal recessive gene Accordingly only 25 per cent of offspring are expected to be affected Apparently sporadic cases are there fore not uncommon Indeed the incidence of sporadic cases in Fanconi s anaemia has been reported to be 25 per cent (7) Assuming the same mode of transmission for the *group with* out congenital malformation a similar inci dence of apparently non familial cases is to be expected Since however congenital malfor mations are absent in these cases the diagnosis of a heredofamilial disorder may be missed *With the help of cytogenetic techniques it may* be possible to classify some idiopathic cases of aplastic anaemia in childhood as belonging to the hereditary *group*

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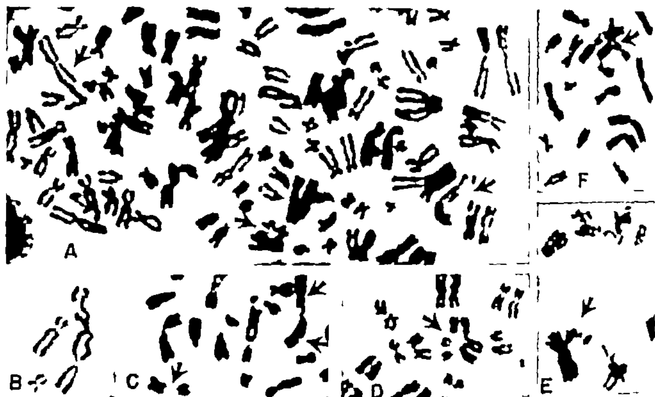



*Fig 1* Chromosomal aberrations found in cases 2 and 3 (*A*) Endoreduplicated metaphase chromatid breaks and gaps chromatid exchange (quadriradial figure) Dicentric chromosome with chromatid break (*left*) (*B*) Dicentric chromosome with break (*C*) Chromatid breaks and gaps (*D*) Chromatid breaks and gaps found in endoreduplicated metaphase (*E*) Multiple chromatid breaks in endoreduplicated cell (*F*) Chromatid exchange (triradial figure)

In many respects with the notable exception of the absence of congenital malformations our patients resembled Fanconi s anaemia The points of similarity will be discussed in the following paragraphs

All our patients were born small for date i e their birth weight was low for their gestational age After birth the boy (O E) followed a growth channel close to the lower limit of normal while the two girls showed a normal pattern Low birth weight and growth retardation have been a common finding in Fanconi s anaemia (5 7)

Fanconi s anaemia is diagnosed relatively late in life usually not before the fifth year This late manifestation of a congenital marrow defect is indeed one of the unsolved problems in Fanconi s anaemia Our oldest patient (E E) presented at 5 1/ years with severe pancytopenia and marked marrow hypoplasia In the younger siblings who had been under closer observation it was possible to follow the gradual decline in the various cell lines from an earlier age At 8 and 4 years respectively they show a relatively mild, slowly progressive pancytopenia

Hyperpigmentation was already noticed by Fanconi (4) in his original cases and has been frequently observed subsequently Areas of brownish pigmentation were present in O E and T E In the older patient (E E) they were not looked for since familial anaemia was not suspected and may have been missed

Haematological features typical of Fanconi s anaemia are an increase in foetal haemoglobin (9) and macrocytosis The latter was present in Fanconi s original cases and led him to describe the anaemia as perniciosiform There was marked macrocytosis in all three patients with the MCV ranging from 100 to 120 cu $\mu$ Foetal haemoglobin was increased to around 10 per cent in the two cases in which it was examined

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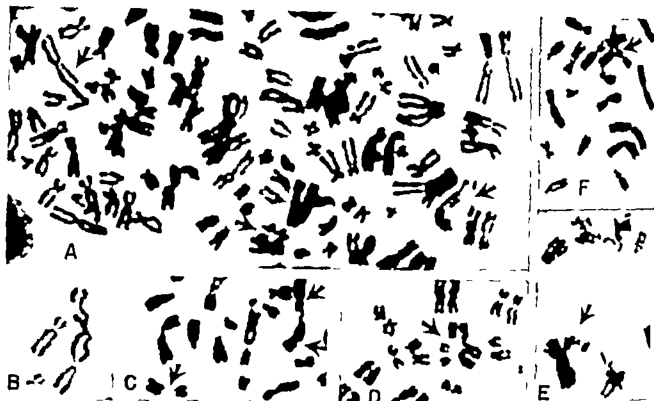



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# SEVERE ILLNESSES DUE TO ADENOVIRUS TYPE 7 IN CHILDREN

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The adenoviruses have been estimated to cause from 1 9 to 2 7 per cent of the acute respiratory illness s among children in their homes In children hospitalized with acute respiratory disease the rate of infections with adenoviruses ranges from 4 1 to 24 8 per cent (19)

Most of the affections caused by these viruses are mild upper respiratory illnesses but sometimes the lower respiratory tract may be involved with bronchitis bronchiolitis or bronchopneumonia The latter and more severe infections have usually been associated with adenovirus types 3 or 7 (16) In 1957 Chany *et al* described four fatal cases among children (5) Subsequently such fatal cases have been reported from different countries (4 10 11 18) but not from Scandinavia

The severe bronchopneumonias due to adenovirus infections are often accompanied by extrapulmonary manifestations (3 5 7 8 11 14 20) They include encephalitis myocarditis renal involvement, hepatomegaly hemorrhagic tendency peripheral edema gastro enteritis and exanthema.

In the following four children aged 1–9 years with severe bronchopneumonia and infections with adenovirus type 7 will be reported In one of the patients the disease was fatal The four patients came from different parts of Norway and no similar diseases were reported in their environments

## CASE REPORTS

The clinical picture and some laboratory results summarized in Tables 1 and 2 Cases no 3 and 4 had pneumonia with few other symptoms while cases no 1 and no 2 revealed several extrapulmonary manifestations as well These two cases will be described in greater detail

*Case 1*

Girl born June 6 1965 The child had previously been healthy On May 6 1966 she had signs of an upper respiratory infection Four days later she developed high fever and signs of bilateral pneumonia were found In spite of penicillin and tetracycline therapy the pneumonia progressed She was admitted to the local hospital on May 15 Chest X ray revealed infiltrations mainly in the right lung Further antibiotic treatment did not improve her condition From May 18 marked peripheral cyanosis was noted Three days later she developed generalized convulsions Steroid treatment was given for 3 days The fever disappeared but the child was weak and soporous CSF was normal On May 24 a generalized bleeding tendency was observed, and blood was present in the stools She was then transferred to the Department of Pediatrics, Rikshospitalet

On admission May 25 her soporous condition persisted The respiration was calm Subcutaneous bleeding and bleeding from needle punctures were noticed She had peripheral edema Pulmonary rales were heard on both sides The liver was enlarged 3–4 cm below the costal margin The day after admission a peculiar redness was observed on the tip of the toes and the fingers One week later the skin detached on the distal parts of several fingers

Examinations on admission revealed thrombocytopenia and derangement of the coagulation factors (Table ) ECG showed low voltage and slight left ventricular preponderance and EEG showed generalized dysrhythmia. Slight albuminuria was present She was treated with vitamin K, antibiotics, plasma and

Table 1 *Clinical and laboratory findings in four children with adenovirus type 7 associated pneum*

| Case no | Age (years) sex | Main clinical signs | Course of disease | Chest x ray | Important laboratory findings | | | EEG |
|---|---|---|---|---|---|---|---|---|
| 1 (J L) | 11/12 F | Pneumonia<br>Hepatomegaly<br>Convulsions and coma<br>Bleeding tendency<br>Edema | 6 weeks duration<br>*Fatal* | Bilateral perihilar infiltrations | 9th day<br><br>20th day | WBC<br>CSF<br>WBC<br>Urea<br>SGOT<br>Serum prot | 9 800<br>normal<br>19 500<br>153 mg/100 ml<br>870 U<br>4 g/100 ml | Generalized dysrythmia |
| | | | | | (See also Table 2) | | | |
| 2 (T C) | 9 M | Pneumonia<br>Encephalitis<br>G-I bleeding | 14 weeks in hospital<br>*Sequelae* | Lobar infiltration right side<br>Persistent middle lobe atelectasis | 6th day<br><br>10th day | WBC<br>SR<br>CSF | 6 900<br>56 mm<br>normal | 6 months later<br>Generalized dysrythmia |
| 3 (S L) | 5 M | Pneumonia | 2 weeks duration | Perihilar infiltration right side | 6th day | WBC<br>CSF | 4 000<br>normal | Not performed |
| 4 (P T) | 1 5/12 M | Pneumonia<br>Meningeal irritation | 3 weeks duration | Dense infiltration right upper lobe | 7th day | WBC<br>CSF<br>Platelets<br>Serum prot | 5 400<br>normal<br>131 000<br>5 3 g/100 ml | Not performed |

blood transfusions and her condition gradually improved The lung infiltrations were less marked and the signs of liver and renal involvement disappeared The coagulation status 18 days after admission showed values above normal for several factors The platelet count was low on several occasions until normal values were reached on the 38th day of illness (Table 2)

Following three weeks of slow improvement her condition suddenly deteriorated with marked respiratory distress cardiac failure fever and leucocytosis She died June 18

Table 2 *Certain hematological findings in case no 1*

| | Day of illness | |
|---|---|---|
| | 20th | 38th |
| Hgb | 12 g/100 ml | 12 7 g/100 ml |
| Platelets | *26 000* | 192 000 |
| PP | 37 | |
| Coagulation factors | | |
| II | 40 | All above 100 |
| V | 230 | |
| VII | 22 | |
| VIII | 250 | |
| IX | 30 | |
| XI | 70 | |
| Fibrinogen | 340 mg/100 ml | 445 mg/100 ml |
| Fibrinolytic activity | — | Increased |

*Case 2*

Boy born Sept 27 1957 He was previously healthy On June 6 1966 he developed a rubella like rash June 16 he got fever and on the next day he had coughing and epistaxis For five days the high fever persisted and did not respond to chloramphenicol therapy He was admitted to the local hospital on June 22 severely ill with high temperature and signs of pneumonia in the right lung confirmed by X ray examination of the chest

A few days later he became soporous with hallucinations CSF was normal Steroid treatment was given for three days in addition to antibiotics On June 30 he developed vomiting and melaena Blood transfusion was given Some few days later the fever subsided and his general condition slowly improved during three months hospitalization The pulmonary findings persisted however with atelectasis of the middle lobe in spite of postural drainage and antibiotics

Six months later the patient was admitted to the Department of Pediatrics Rikshospitalet for further evaluation The clinical and X ray findings of the lung persisted more than one year In September 1967 a middle lobe lobectomy was performed

The EEG registration has been abnormal with generalized dysrythmia on several occasions following the acute disease but there are no clinical signs of brain damage

*Virological findings*

The results of the virological studies are summarized in Table 3

*able 3 Virological findings in 4 children with enovirus – 7 associated pneumonia*

| ase no | Virus isolation: Specimens examined | Days after onset | Result (adeno-virus type 7) | Serological examination: Days after onset | CFT titer |
|---|---|---|---|---|---|
| | Feces | 21 | + | 21 | <1 10 |
| | Throat swab | 1 | – | 32 | 1 80 |
| | CSF | 21 | – | 37 | 1 40 |
| | Feces | 18 | + | 17 | 1 80 |
| | | | | 48 | 1 1280 |
| | | | | 217 | 1 1 80 |
| | | | | 275 | 1 640 |
| | | | | 331 | 1 370 |
| 3 | Feces | 7 | + | 7 | 1 80 |
| | Throat swab | 7 | – | | |
| | CSF | 7 | – | 17 | 1 320 |
| 4 | Feces | 10 | + | 10 | <1 5 |
| | Throat swab | 10 | + | 43 | 1 20 |

Adenovirus type 7 was isolated from the feces of all four patients. A four fold or greater rise in the titre of complement fixing antibodies showed infection with an adenovirus to have taken place. Virus was recovered from one out of three throat specimens but the specimens were taken somewhat late in the course of the diseases. Attempts to isolate virus from CSF were unsuccessful. From the fatal case no autopsy specimen was virologically examined. Virus was not recovered from the lung segment removed from case no. 2 (15 months after the acute illness).

No other viruses were isolated from the patients. Complement fixation tests showed no significant rise in antibody titre with antigens from respiratory syncytial virus or *Mycoplasma pneumoniae*.

Pathogenic bacteria were not recovered from any of the four patients. Toxoplasma dye test was negative.



*Fig. 1.* Proliferation and necrosis of bronchial epithelium. Numerous epithelial cells contain basophilic intranuclear inclusions. Stained with haematoxylin and eosin. × 220.

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| | | | | | (See also Table 2) | | | |
| 2 (T C) | 9 M | Pneumonia<br>Encephalitis<br>G-I bleeding | 14 weeks in hospital<br>*Sequelae* | Lobar infiltration right side<br>Persistent middle lobe atelectasis | 6th day<br><br>10th day | WBC<br>SR<br>CSF | 6 900<br>56 mm<br>normal | 6 months later Generalized dysrythmia |
| 3 (S L) | 5 M | Pneumonia | 2 weeks duration | Perihilar infiltration right side | 6th day | WBC<br>CSF | 4 000<br>normal | Not performed |
| 4 (P T) | 1 5/12 M | Pneumonia<br>Meningeal irritation | 3 weeks duration | Dense infiltration right upper lobe | 7th day | WBC<br>CSF<br>Platelets<br>Serum prot | 5 400<br>normal<br>131 000<br>5 3 g/100 ml | Not performed |

blood transfusions and her condition gradually improved The lung infiltrations were less marked and the signs of liver and renal involvement disappeared The coagulation status 18 days after admission showed values above normal for several factors The platelet count was low on several occasions until normal values were reached on the 38th day of illness (Table 2)

Following three weeks of slow improvement her condition suddenly deteriorated with marked respiratory distress cardiac failure fever and leucocytosis She died June 18

Table 2 *Certain hematological findings in case no 1*

| | Day of illness | |
|---|---|---|
| | 20th | 38th |
| Hgb | 12 g/100 ml | 12 7 g/100 ml |
| Platelets | 26 000 | 192 000 |
| PP | 37 | |
| Coagulation factors | | |
| II | 40 | All above 100 |
| V | 230 | |
| VII | 22 | |
| VIII | 250 | |
| IX | 30 | |
| XI | 70 | |
| Fibrinogen | 340 mg/100 ml | 445 mg/100 ml |
| Fibrinolytic activity | — | Increased |

*Case 2*

Boy born Sept 27 1957 He was previously healthy On June 6 1966 he developed a rubella like rash June 16 he got fever and on the next day he had coughing and epistaxis For five days the high fever persisted and did not respond to chloramphenicol therapy He was admitted to the local hospital on June 22 severely ill with high temperature and signs of pneumonia in the right lung confirmed by X ray examination of the chest

A few days later he became soporous with hallucinations CSF was normal Steroid treatment was given for three days in addition to antibiotics On June 30 he developed vomiting and melaena Blood transfusion was given Some few days later the fever subsided and his general condition slowly improved during three months hospitalization The pulmonary findings persisted however with atelectasis of the middle lobe in spite of postural drainage and antibiotics

Six months later the patient was admitted to the Department of Pediatrics Rikshospitalet for further evaluation The clinical and X ray findings of the lung persisted more than one year In September 1967 a middle lobe lobectomy was performed

The ELG registration has been abnormal with generalized dysrythmia on several occasions following the acute disease but there are no clinical signs of brain damage

*Virological findings*

The results of the virological studies are summarized in Table 3

able 3 *Virological findings in 4 children with t enovirus – 7 associated pneumonia*

| | Virus isolation | | | Serological examination | |
|---|---|---|---|---|---|
| Case no | Specimens examined | Days after onset | Result (adeno-virus type 7) | Days after onset | CFT titer |
| 1 | Feces | 21 | + | 21 | <1 10 |
| | Throat swab | 21 | – | 37 | 1 80 |
| | CSF | 21 | – | 37 | 1 40 |
| 2 | Feces | 18 | + | 17 | 1 80 |
| | | | | 48 | 1 1280 |
| | | | | 217 | 1 1280 |
| | | | | 225 | 1 640 |
| | | | | 331 | 1 320 |
| 3 | Feces | 7 | + | 7 | 1 80 |
| | Throat swab | 7 | – | | |
| | CSF | 7 | | 17 | 1 320 |
| 4 | Feces | 10 | + | 10 | <1 5 |
| | Throat swab | 10 | + | 43 | 1 20 |

Adenovirus type 7 was isolated from the feces of all four patients. A four fold or greater rise in the titre of complement fixing antibodies showed infection with an adenovirus to have taken place. Virus was recovered from one out of three throat specimens but the specimens were taken somewhat late in the course of the diseases. Attempts to isolate virus from CSF were unsuccessful. From the fatal case no autopsy specimen was virologically examined. Virus was not recovered from the lung segment removed from case no. 2 (15 months after the acute illness).

No other viruses were isolated from the pa tients. Complement fixation tests showed no significant rise in antibody titre with antigens from respiratory syncytial virus or *Mycoplasma pneumoniae*.

Pathogenic bacteria were not recovered from any of the four patients. Toxoplasma dye test was negative.



Fig. 1. Proliferation and necrosis of bronchial epithelium. Numerous epithelial cells contain basophilic intranuclear inclusions. Stained with haematoxylin and eosin. 220

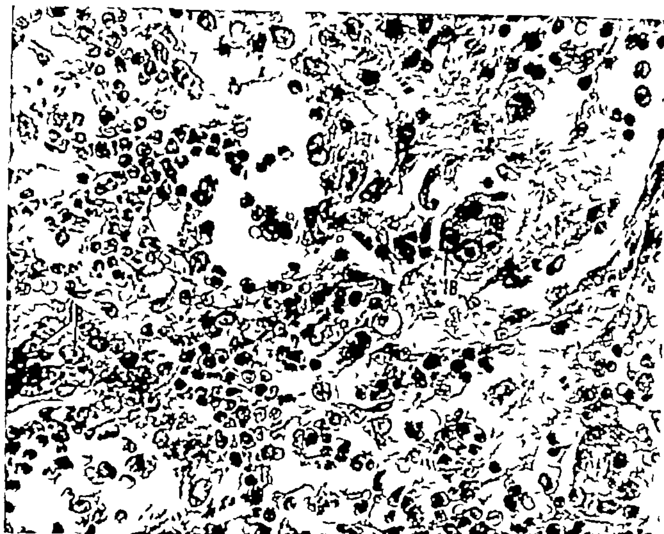



*Fig 2* A section of lung demonstrating greatly en larged alveolar cells Many of the proliferating cells showed swollen nuclei which frequently contained basophilic inclusion bodies (IB) Stained with haematoxylin and eosin × 880

*Pathological findings*

*Case no 1* The major pathological findings were confined to the lungs Both were heavy and dark red in colour There were patches of consolidation, particularly in the lower lobes Microscopic examination revealed a broncho pneumonia characterized by mononuclear cell infiltration Occasionally small necrotic areas were observed and sometimes the alveoli also contained fibrin or blood Polynuclear cell in filtration was very rarely seen Most often it was present in the necrotic areas In the bronchial mucosa areas of necrosis and degeneration were frequently found A predominant finding was the proliferation of the bronchial and alveolar epithelium (Fig 1) In the alveoli the proliferation was particularly marked in the areas of lung parenchyma surrounding the bronchi Many of the proliferating cells were greatly enlarged Moreover the nuclei were swollen and frequently contained basophilic in clusions In some of the affected cells the basophilic masses were surrounded by clear zones (Fig 2) Only occasionally were the inclusions eosinophilic Cytoplasmic inclusions were not observed No bacteria or fungi were demonstrated by special stains

The brain showed edema and some meningeal and perivascular infiltration of mononuclear cells In the hippocampus regions degeneration of the nerve cells and a considerable glial reaction were found No specific cytological changes were observed

The liver showed fatty changes and cloudy

swelling. In some areas necrosis with calcification was found. In the spleen small fibrotic areas were present.

Fibrin thrombi in the small vessels were not observed in any organ.

*Case no. 2*. Apart from a diffuse atelectasis and fibrosis microscopic examination of the middle lobe showed a chronic inflammation of the bronchial wall. A considerable infiltration of lymphocytes and plasma cells was present, particularly in the submucosa. Moreover a remarkable proliferation of the bronchial epithelium was observed.

## DISCUSSION

The virological examination showed that all patients had had a current adenovirus type 7 infection during the observation period. In cases nos. 2, 3 and 4 the onset of their adenovirus infection and the onset of the illness seemed to coincide in time. Patient no. 1 however could have been infected by adenovirus during an illness caused by an unidentified agent. Dual infections are not uncommon in infants (12). On the other hand the complement fixation antibody response in infants may sometimes be weak and the complement fixation test is not a very sensitive test for detecting adenovirus antibodies in this age group (12).

All four patients had pneumonia with high fever lasting for ten days or more and did not respond to antibiotic treatment. Leucocytosis was not present initially, a finding which aroused suspicion of virus etiology. The X-ray findings did not point to a specific etiology. No characteristic roentgenological patterns have been described earlier and in one series of patients with adenovirus pneumonitis both perihilar and dense lobar or lobular infiltrations were found (8). The long lasting pulmonary involvement demonstrated by case no. 2 has been observed by others (8, 20).

The course of the disease was favourable in two of our patients, prolonged in case no. 2 and fatal in case no. 1. This patient succumbed in a cardiopulmonary hypoxic collapse after a period of improvement. A similar diphasic course in the fatal disease has also been noted by others (3, 5, 8). It is uncertain whether this sudden deterioration in the patient's condition was due to the adenovirus infection or whether a secondary bacterial infection was involved. The patient was still on continuous antibiotic therapy when she died.

Cases no. 1 and no. 2 had signs of cerebral affection with convulsions and altered consciousness. Case no. 4 had a slight neck stiffness and resisted feedings for severel days. A pathological EEG registration was found in the two patients examined. In all four patients the cerebrospinal fluid was normal. Meningoencephalitic manifestations are reported to occur rather frequently in children with severe pneumonia due to adenovirus type 7. Mallet *et al.* (8) found that 12 out of 18 patients showed signs such as reduced consciousness, convulsions, hypotonia and nystagmus. Late sequelae as in our case no. 2 have been observed before (20).

The pathogenesis of the cerebral affections in patients infected with adenovirus has not been settled (14). In our fatal case the brain pathology suggested extensive hypoxia.

Children with severe pneumonia may have an enlarged liver as sign of cardiac failure. Liver enlargement was prominent in case no. 1 at a time when respiratory symptoms were less marked. Several of the reported cases of fatal and severe adenovirus 7 infections had hepatomegaly probably not related to cardiac insufficiency (3, 4, 8, 10). The low serum protein values in two of our cases, the reduction of the coagulation factors which are produced in the liver and the increased transaminase value in case no. 1 all indicate involvement of the liver.

Several authors have observed a bleeding tendency in severe infections with adenovirus type 7 (3, 8, 10), but the mechanism of bleeding remains obscure. McKay *et al.* (9) have claimed that intravascular coagulation occurs in several virus diseases. Depending on the

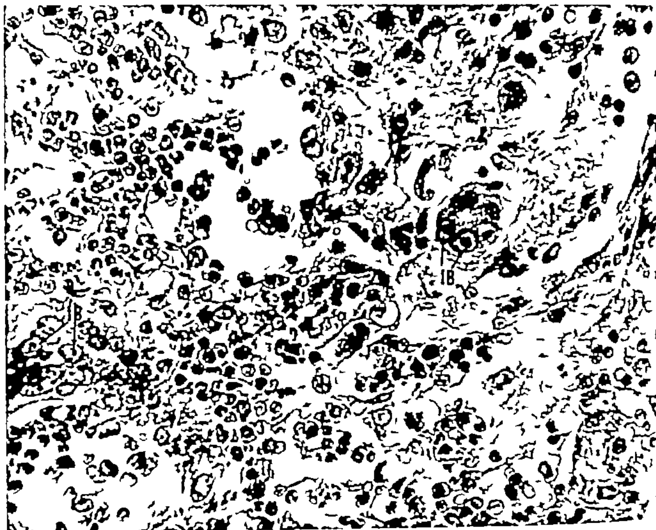



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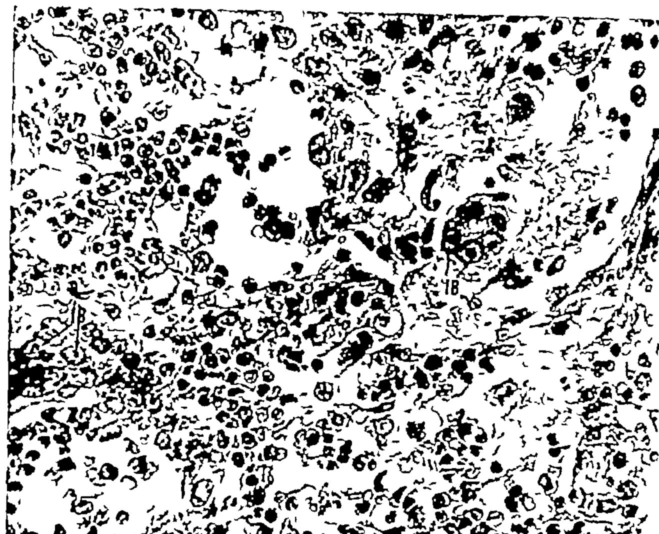



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location of the intravascular thrombi many and different symptoms and signs may be the result of the coagulation process The depletion of coagulation factors is followed by a period of overproduction when coagulation has subsided

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Von Zuane *et al* (20) and others (3) have described vascular changes with edema, cyanosis and skin necrosis in children with severe infections due to adenovirus type 7 Similar signs were demonstrated in the fatal case presented The edema did not seem to be related to a cardiac failure The low serum protein values combined with a slow peripheral circulation possibly caused by intravascular changes, may explain the cyanosis as well as the edema and skin necrosis

Several of the extrapulmonary symptoms and signs discussed here might be explained by a vascular disorder initiated by this particular virus infection intravascular coagulation may play a part in the pathogenesis We did not give heparin but such treatment of intravascular coagulation occurring during other diseases seems to be of value Therefore similar cases in the future should be thoroughly investigated hematologically and heparin treatment should be considered

## SUMMARY

Four patients presenting a severe pneumonia due to adenovirus type 7 are reported One of them died, and intranuclear inclusion bodies characteristic for the illness were found In infants and small children this infection might in addition give meningoencephalitic symptoms, hepatomegaly vascular disorders and hemorrhagic tendency as some of these patients demonstrated Intravascular coagulation initiated by the virus infection could explain several of the symptoms and signs in such serious cases

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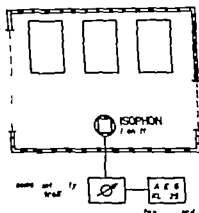
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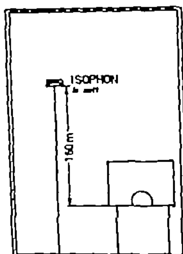




*Fig 1* Plan and profile of the test room with sound box tape recorder and noise intensity controller

## METHODS

The wake up threshold was studied in 126 healthy or convalescent probands of 3–63 weeks of age. Two-thirds of these were male. All acoustic wakening tests were performed in the night hours between 10 30 p.m and 01 00 a.m. Sleep-promoting drugs were excluded from all children. A precise record of the wakening effect by means of EEG tracings was abandoned as the necessary manipulations might have influenced the falling asleep and thus the sleep/wake phases from the beginning. The criterion of the required deep sleep before each test was the observation of the usual resting sleep position of the infant. The spontaneous giving up of this position was taken as a sign of a disturbing effect. Quick reactions towards the environment and contacts with it were identified with the waking state.

The noise exposure was regularly performed in the same room gauged for these investigations (Fig 1). A tape recorder (AEG type KL 25) was used as the noise source. This was placed outside of the test room to avoid the set noises during the test and to exclude the sharp switch-on click from the test room. The sound stimulus used had the character of a factory noise with a frequency range of 100–7000 Hz. The acoustic analysis is shown in Fig 3. The noise tape revolved in about 8 seconds the reproduction was uninterrupted. The noise stimulus was emitted into the test room by a sound box (type Isophon Isonetta) fixed 1 6 m above the proband's head. The emission time had a maximum of 12 minutes. If the proband woke up earlier this time as well as that of the first signs of disturbance were taken and the test stopped. Each proband was exposed only once and to only one consistent sound intensity of 50 55 60 65 70 or 75 dB during one night. By repeated studies in different nights the wake up threshold i.e. the noise level necessary to wake up from sleep could finally be determined. To preclude additional acoustic or other alarm-effects the examiner observed the proband from outside the test room through a window with constantly low and indirect light.

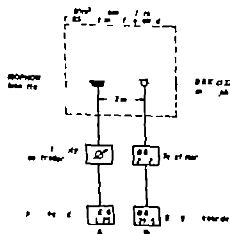



*Fig* Diagram of connections for gauging noise intensities of tape recorder/sound box set (A) and for testing the noise level in different types of sick rooms (B)

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*Acta Pædiat Scand 58* 164–170 1969

# THE NOISE LEVEL IN A CHILDRENS HOSPITAL AND THE WAKE UP THRESHOLD IN INFANTS

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*From the Children s Hospital (Head W Künzer) and the Hospital for Ear Nose Throat Diseases (Head F Zollner) University of Freiburg/Br Germany*

The sleeping periods of infants during the first two months of life are confined to single phases of at most 3 hours and fairly regularly dis tributed throughout day and night At an age of 3 months a more and more distinct day–night rhythm develops with continuously increasing periods of waking and sleeping After 6 months the infant is awake almost exclusively during daytime

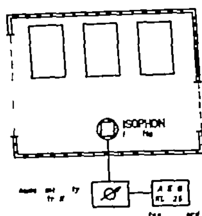
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1 What environmental noise intensity en dangers the infant s sleep?
2 What is the environmental noise level in the present type of construction and or ganisation of work in care units of a chil dren s hospital?

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Investigations supported by the Deutsche Forschungs gemeinschaft



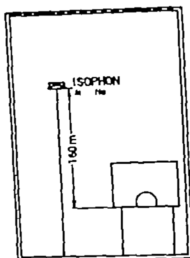




*Fig. 1.* Plan and profile of the test room with sound box, tape recorder and noise intensity controller.

## METHODS

The wake up threshold was studied in 126 healthy or convalescent probands of 3–63 weeks of age. Two-thirds of these were male. All acoustic wakening tests were performed in the night hours between 10.30 p.m. and 01.00 a.m. Sleep-promoting drugs were excluded from all children. A precise record of the wakening effect by means of EEG tracings was abandoned as the necessary manipulations might have influenced the falling asleep and thus the sleep/wake phases from the beginning. The criterion of the required deep sleep before each test was the observation of the usual resting sleep position of the infant. The spontaneous giving up of this position was taken as a sign of a disturbing effect. Quick reactions towards the environment and contacts with it were identified with the waking state.

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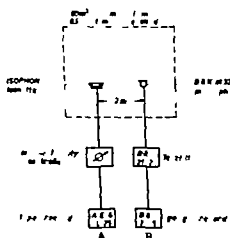



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2 What is the environmental noise level in the present type of construction and organisation of work in care units of a children s hospital?

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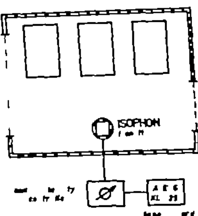



Fig 1 Plan and profile of the test room with sound box tape recorder and noise intensity controller

## METHODS

The wake up threshold was studied in 126 healthy or convalescent probands of 3–63 weeks of age Two-thirds of these were male All acoustic wakening tests were performed in the night hours between 10 30 p.m and 01 00 a m Sleep-promoting drugs were excluded from all children A precise record of the wakening effect by means of EEG tracings was abandoned as the necessary manipulations might have influenced the falling asleep and thus the sleep/wake phases from the beginning The criterion of the required deep sleep before each test was the observation of the usual resting sleep position of the infant The spontaneous giving up of this position was taken as a sign of a disturbing effect Quick reactions towards the environment and contacts with it were identified with the waking state

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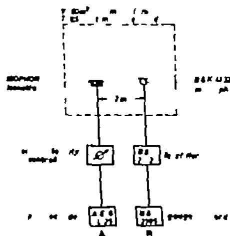



Fig 2 Diagram of connections for gauging noise intensities of tape recorder/sound box set (A) and for testing the noise level in different types of sick rooms (B)

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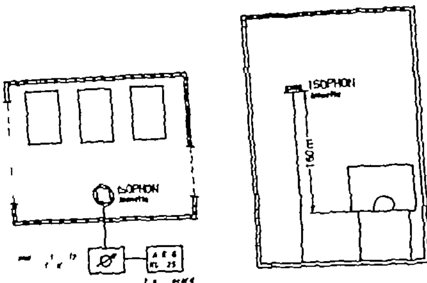



*Fig 1* Plan and profile of the test room with sound box tape recorder and noise intensity controller

## METHODS

The wake up threshold was studied in 176 healthy or convalescent probands of 3–63 weeks of age Two-thirds of these were male All acoustic wakening tests were performed in the night hours between 10 30 p m and 01 00 a m Sleep-promoting drugs were excluded from all children A precise record of the wakening effect by means of EEG tracings was abandoned as the necessary manipulations might have influenced the falling asleep and thus the sleep/wake phases from the beginning The criterion of the required deep sleep before each test was the observation of the usual resting sleep position of the infant The spontaneous giving up of this position was taken as a

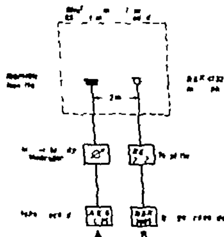



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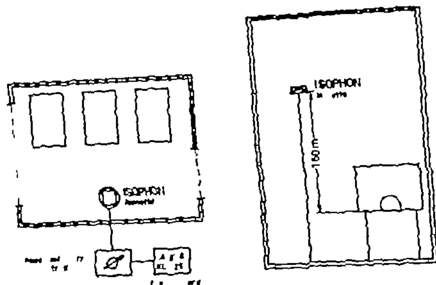



Fig 1 Plan and profile of the test room with sound box tape recorder and noise intensity controller

## METHODS

The wake up threshold was studied in 126 healthy or convalescent probands of 3–63 weeks of age. Two-thirds of these were male. All acoustic wakening tests were performed in the night hours between 10 30 p m and 01 00 a m. Sleep-promoting drugs were excluded from all children. A precise record of the wakening effect by means of EEG tracings was abandoned as the necessary manipulations might have influenced the falling asleep and thus the sleep/wake phases from the beginning. The criterion of the required deep sleep before each test was the observation of the usual resting sleep position of the infant. The spontaneous giving up of this position was taken as a sign of a disturbing effect. Quick reactions towards the environment and contacts with it were identified with the waking state.

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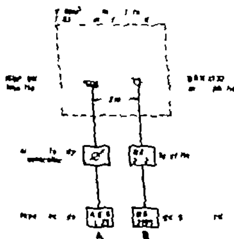



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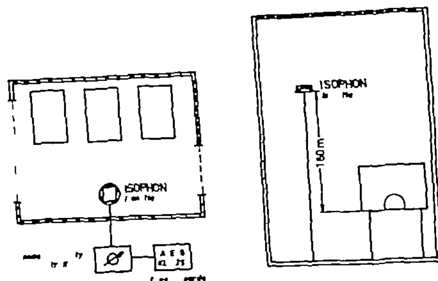



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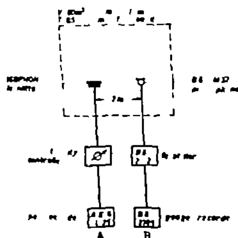



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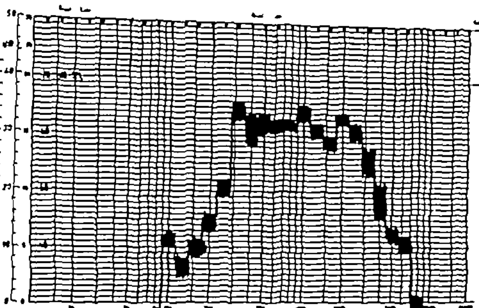


*Fig 3* Acoustic terz analysis of used noise tape (paper speed 0 1 mm/sec)

sec (Fig 3) The sapphire needle used in the recordings was moved according to the moving coil principle the speed of the engraving tool was 16 mm/sec

The gauging proved a limit of error in sound emission of 4 dB This drawback is unavoidable in employing a sound box emission The acoustic condition of the test room strongly interferes with the noise level of the proband's positional area The great advantage of the method however lies in the fact that the proband falls asleep undisturbed which cannot be presumed using earphones that mostly render the investigations impossible

The noise level testing *in different types of sick rooms* was performed in dB (linear) and DIN phone over periods of 24 hours each As with the described gauging procedures of the noise source the diffuse sound field of each room was registered by all around sound recording also using a microphone without regional character (type Bruel & Kjaer No 4132) The impulse registration took place irrespective of frequency with a spectrometer (type Bruel & Kjaer No 2112) and gauge recorder as well The speed of the waxed paper strip was set to approx 1 cm/hour The pencil moved with a speed of 250 mm/sec A noise to be registered in true volume had to last at least 0 25 sec according to DIN 5045 (IEC) As the recording sapphire sets a finite stroke breath the maximal time resolving of impulses with this kind of registration is about 30 sec

The interpretation of measuring stripes was achieved by taking 3 noise magnitudes (example Fig 4)

(a) *Basic noise* This included all sound impulses that had been permanently registered by the recorder more often than each half minute

(b) *Medium peak noise* Thus was termed the average noise maximum registered within the test room at least 15 to 18 times per hour

(c) *Absolute peak noise* This was evidenced by the single maximum sound volumes in each time period

## RESULTS

The waking noise thresholds registered with 126 infants are shown comprehensively in Fig 5 *b* and *c* It is evident that almost ¹/ to /₃ of the infants were disturbed in sleep—partially to awake—after 3 min by mixed noise between c 100–7000 Hz at a sound level of 70–75 dB A noise exposition of this type of more than 12 minutes disturbed more than ¹/₃ of the probands at a intensity of 65 dB A noise level of

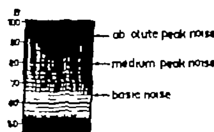



*Fig 4* Diagram of basic noise medium peak and absolute peak noise in different care sick rooms

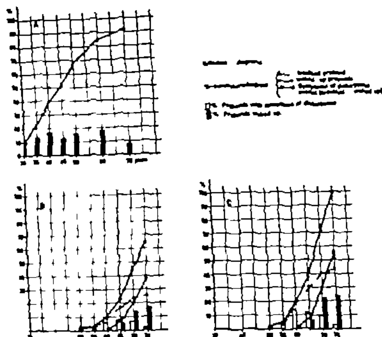


*Fig. 5.* Minimal noise levels of troubling and wake up thresholds. (A) In adults, noise lasting 3 min (Steinicke). (B) In infants, noise lasting 3 min (own investigations). (C) In infants, noise lasting 12 min (own investigations).

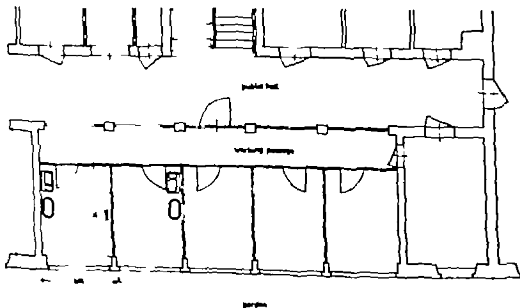


*Fig. 6.* Baby units. Material of walls: steel frame. Floor: Spoknol; ceiling: plaster.

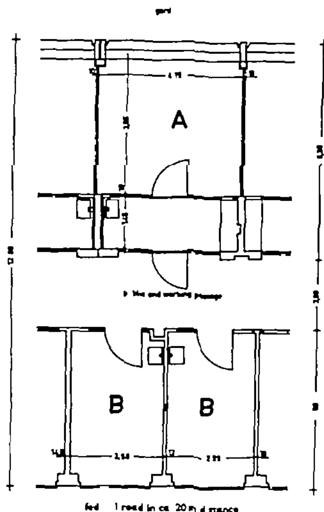



*Fig 7* Children s unit (A) Rooms faced to garden with sluice Walls Wood artificial stone glass floor Spoknol ceiling plaster (B) Rooms faced to federal road Walls brick stones floor Spoknol ceiling *plaster*

75 dB consistently causes sleep disturbance or awaking The process of waking up was almost invariably characterized by a startle reflex when noise volumes of 75 dB—sometimes even 70 dB —were applied This appeared with the onset of noise and subsided quickly No differences worth mentioning were found in infants of dif ferent ages The study of the different noise levels in different types of sick rooms demonstrated a relationship between noise volume and type of construction In so called infant units with glass walls in steel frames and a working passage in front of these (Fig 6) as well as a window row to a garden *aside of street noise* the *basic noise* at night did not appreciably reach less than 50 dB during daytime it varied between 60 and 70 dB The average peak noise was registered at night at about 80 dB, during the day it was about 90 dB Maximum single impulses exceeded the 90 dB limit throughout the 24 hour period, these fall into the "absolute noise zone (Fig 8 I)

Sick rooms also facing a garden but with wall constructions of artificial stone wood and glass and with a desinfection sluice in front made of the same material (Fig 7 *a*) proved to have comparatively a somewhat lower basic noise (50 dB) average peak noise (60–70 dB) and maximal single noise (70–80 dB) at night During daytime the noise intensities, however corresponded to those in rooms with steel-glass walls (Fig 8 II *a*)

## DISCUSSION

The presented results of this study demonstrate that an acoustic disturbance of some minutes duration at a level greater than 70 dB in every day noise frequencies (*c* 100–7000 Hz) is in compatible with an infant s sleep Wedenberg (9) came to the same results in audiograms of 20 newborns These infants were roused from deep sleep by sine tones of 500–3000 Hz at a sound level of 70–75 dB and from shallow sleep at about 55 dB Changes of breathing type as an indication of sleep disturbance were observed by Rosenau (7) and Lehnhardt (5) in pre school children exposed to noises of 50 dB A com parison of such thresholds of rousing noise effects determined in infants and small children with analogous effects in men of older age (Steinicke (8 Fig 5 *a*) ) indicates that the in fants sleep is less disturbable by acoustic stimuli than that of adults Thus the empirical *concep* tion of the sound sleep of children is *confirmed* The recordings of noise levels have shown how ever that average noise in care units of a chil dren s *hospital nevertheless exceed the high* tolerance of the infant s sleep This applies par ticularly to rooms whose construction was con *ceived for consistent control by the nursing* staff with constructional elements mainly of steel and glass The demonstrated differences in th[illegible]

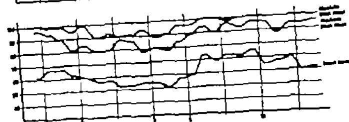
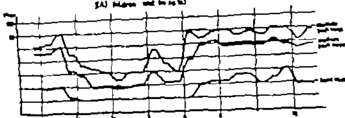
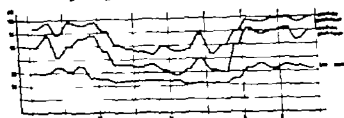





Fig 8 Statistical diagrams of basic noise medium peak noise and absolute peak noise in a baby care unit in a children's-care box and in a children's sick room

levels of different room types and room positions indicate in addition that the "interior clinic noise" operates more intensely than noises from outside

Indeed experience shows that infants sleep even under these bad acoustic conditions Probably one reason among others is to be seen in the fact that the intensity of noises measured by us may not be identified with continuous noise stimuli of this intensity The time breaking of the recording unit set to about 30 seconds implies this restriction The total of noise impulses exceeding the tolerance limit demonstrates however a serious deficiency of pediatric hospital hygiene It has to be fought by improvements of construction and mode of working

## SUMMARY

One hundred and twenty six children 3 to 63 weeks of age have been exposed to a mixed noise (100–7000 Hz) in intensities between 50 and 80 dB under steady conditions of room acoustic during different periods of time between 10 30 p m and 01 00 a m A noise level of 75 dB led to obvious sleep disturbance or waking up in /3 of the children after 3 minutes

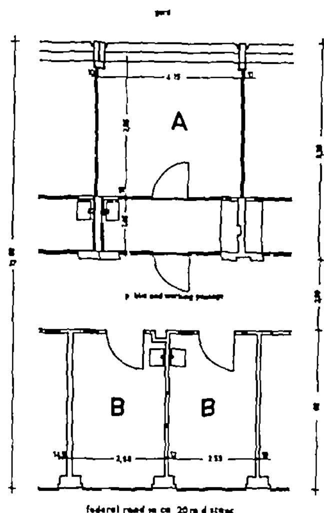



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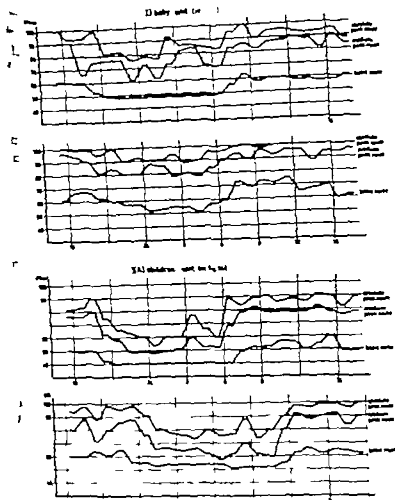


Fig. 8. Statistical diagrams of basic noise, medium peak noise and absolute peak noise in a baby care unit, in a children's-care box and in a children's sick room.

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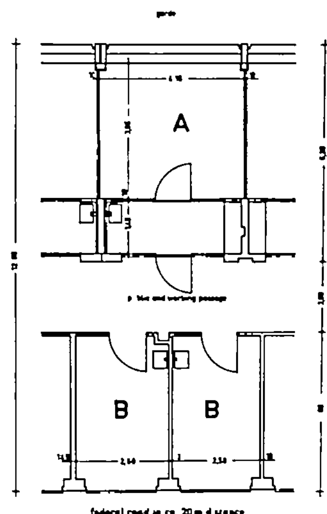



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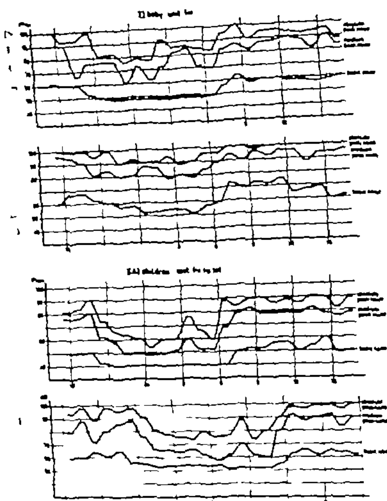


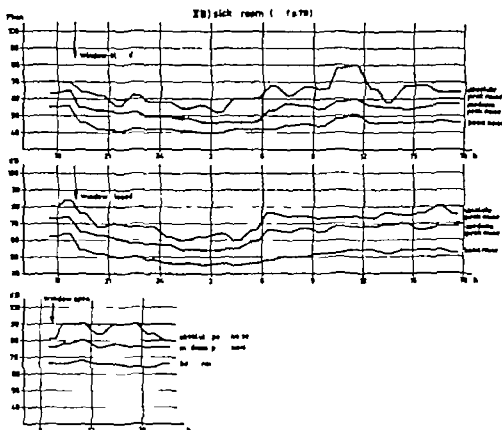
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and in all after 12 minutes The waking threshold according to these investigations is higher than in adults

A 24-hour recording of the noise level in different types of infants and children's care units demonstrated that the acoustic stress—especially by interior working noise —in care units of light construction reaches or exceeds the obligate waking noise threshold of infants during most of the day and night hours This is regarded as a deficiency of hospital hygiene

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9 Wedenberg E Objective auditory tests on non cooperative children *Acta Otolaryng* (Stockholm) Suppl *175* 1963


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*Key words* Infants noise level wake up threshold childrens hospital

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# REMOVAL OF INDIRECT REACTING BILIRUBIN BY ALBUMIN BINDING DURING INTERMITTENT PERITONEAL DIALYSIS IN THE NEWBORN

NIELS HOBOLTH and MOGENS DEVANTIER

*From the Pediatric Department Gentofte Hospital (Head P W Braestrup) Hellerup Denmark*

In neonatal jaundice not caused by blood group incompatibility simple removal of bilirubin should be sufficient treatment to avoid kernicterus In 1962 Grollman & Odell (3) demonstrated that measurable amounts of indirect reacting bilirubin as well as direct reacting bilirubin could be extracted by peritoneal dialysis when the dialysing fluid contained albumin Later Shoshkes *et al* (6) compared extracorporeal hemodialysis and peritoneal dialysis with and without albumin in dialysing fluid They used dogs made hyperbilirubinaemic by ligation of the common bileduct They doubt the possibility of removing significant amounts of bilirubin by peritoneal dialysis

## METHODS

With suitable methods for peritoneal dialysis on infants and children in hand (1 5) we felt inclined to judge the value of protein peritoneal dialysis in treating neonatal jaundice not caused by blood group incompatibility

As described in details elsewhere ( ) we use a Medi Plast® cannula 15 G (outer diameter 1 8 mm) with a 15 G steel cannula the plast cannula being 60 mm in length and with several perforations in the distal 3 mm made in advance by a morphia cannula

Through this cannula amounts preferably corresponding to 50 ml infusion fluid per kg body weight are instilled After thorough sterilization at a point about midway between umbilicus and left anterior superior iliac spine After incision the Medi Plast® cannula is introduced through a small cutaneous incision in the midline of the abdominal wall at a point below the umbilicus and lying / of the distance between this and the symphysis The individual layers are displaced as much as possible during the insertion. When the tip of the plast cannula is just inside the peritoneal membrane the inner steel cannula is removed and the plast cannula is lodged in the fossa Douglasi.

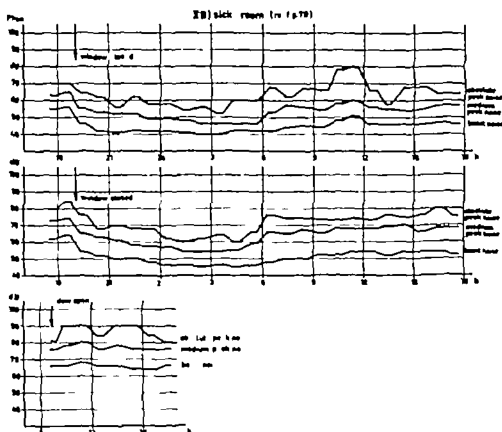
Dialysis was performed on a newborn premature boy (birthweight 1600 g) on his fourth and seventh day of life Each dialysis consisted of 2 installations lasting each 4 hours giving a total dialysing time of 16 2 hours There were no complications

The dialysing fluid consisted of a stock solution of 9 % protein prepared by the Danish State Serum Institute and diluted by equal parts of an electrolyte solution (Table 1) Addition of 1.5 % glucose to the first installation evidently gave a too high osmolality causing a considerable increase in the child's PCV (Packed Cell Volume)

Fig 1 shows the relevant data All bilirubin estimations have been made by a spectrophotometric method (4) It is seen that the concentration of bilirubin in the dialysing fluid increases along a nearly straight line as a function of time A total of 10 83 mg bilirubin was extracted in 16 2 hours To facilitate calculations a mean serumbilirubin of 21 mg/100 ml was taken (cf Fig 1) With a bilirubin "space" equal to 4 times plasmavolume (7) total body bilirubin is 43 8 mg As mean bilirubin extraction is 0 67 mg/hour or 1.2 % per hour 57 9 hours are needed to lower bilirubin concentration to half the original value Calculations on the data presented by Grollman & Odell (3) give the same T/2 for indirect reacting bilirubin (Table 2)

## DISCUSSION AND CONCLUSION

Our results are in full agreement with the findings of Grollman & Odell (3) Shoshkes *et al* (6) have found a very low bilirubin extraction and even so after the admixture of albumin to the dialysing fluid This is almost certainly



and in all after 12 minutes The waking threshold according to these investigations is higher than in adults

A 24 hour recording of the noise level in different types of infants and children's care units demonstrated that the acoustic stress—especially by interior working noise —in care units of light construction reaches or exceeds the obligate waking-noise threshold of infants during most of the day and night hours This is regarded as a deficiency of hospital hygiene

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# SECONDARY CYSTATHIONINURIA

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Increased urinary excretion of cystathionine has been reported in 6 patients and was associated in 5 of them with mental retardation. This patient group is interpreted as having an inborn error of metabolism due to a disturbance in the metabolic breakdown of cystathionine (primary cystathioninuria) (4). Several reports of increased urinary excretion of cystathionine in children in combination with various diseases eg pyridoxine deficiency neuroblastoma galactosaemia and hepatoblastoma have been published (for review of such cases of secondary cystathioninuria see Shaw *et al*) (11).

The purpose of the present investigation was to study the frequency of cystathioninuria in larger groups of hospitalized children.

## MATERIAL AND METHODS

The material e mined consists of 30 children in whom the urinary aminoacid pattern had been requested on the ground of suspected metabolic disorders, especially in patients with malformation mental retardation or convulsions. The patients were all hosp tal zed at the paediatric department General Hospital Malmo. The urine samples from patients had been st red at 4 and were analysed within 3 days.

The urinary amino nitrogen were measured as described by Khachadurian *et al* (8). The urinary aminoacids were separated by high-voltage paper electrophoresis combined with paper chromatography (13). Samples of urine which contained 5 mg of creatinine were initially poured through a 3 cm c lumn of Zeo-Karb 25 resin to remove urea and to reduce volume (7). The concentrated aminoacid solution corresponding to 75 μg creatinine was then applicated to Whatman No 3 MM paper and separated by electrophoresis with formic-acetic acid pH = 1 2. The voltage gradient was 40 V per cm running time 90 minutes. Three parallel runs were performed on the same paper. After electrophoresis two strips were cut away from the large sheet of paper and were stained with ninhydrin (12) iodine platinate (12) respectively. The last reagent was used for localizing the sulphur-containing aminoacids. The third strip was used for chromatography in the second dimension ( butanol acetic acid, water 12 3 5 v/v) over night. Under the electrophoretic conditions described methionine and cystine migrated together but cystathionine had a greater migration rate towards the cathode. The only sulphur-containing aminoacid which had the same migration rate as cystathionine under these conditions was homocystine. Chromatography in the second dimension separates these two aminoacids as well as methionine and cystine. The following Rf values were found Cystine = 0 08 methionine = 0,52 homocystine 0 18 and cystathionine = 0 10.

Homocystine was never found. The sensitivity of the iodineplatinate reagent allows detection of cystathionine in concentrations as low as 0 2 μg per spot. The recovery of cystathionine by this method was about 90% thus the lower limit of detection was about 2 mg cystathionine per g creatinine.

When a sulphur positive aminoacid in the area of cystathionine was detected the chromatographic behaviour in at least 3 different solvent systems (methanol water pyridine 16 4 8 n-butanol pyridine water 1 1 1 t-butanol water methyl ethyl ketone diethylamine 80 80 40 8) was checked and co-chromatography performed to prove the identity. In addition cystathionine was oxidized to its sulphoxide form by means of hydrogen peroxide and its chromatographic behaviour checked. When cystathionine could be demonstrated the electrophoretic run of the urine extract was repeated together with appropriate standards of authentic l-cystathionine and compared visually. The accuracy of this semiquantitative

Table 1 *Composition of stock solutions and dialysing fluid*

| | SS protein concentrate C-17 | Dilution fluid | Dialysing fluid |
|---|---|---|---|
| Na mEq/l | 123 | 157 | 140 141 |
| K | 2 6 | 5 4 | 4–4 3 |
| Ca | 7 6 | 0 | |
| Mg | 2 4 | 0 6 | |
| Cl | 27 | 119 | 67–77 |
| $HCO_3$ | 6 | 44 | 22 |
| P mg/100 ml | 2 | 0 | |
| Protein g/l | 104 | 0 | 52–59 |
| Osmolality mOsm/l | | | 365–385 |
| Glucose (added) g/l | | | 15 |
| Osmolality mOsm/l | | | 405 |

explained by the fact that they were examining dogs who were jaundiced almost exclusively from direct reacting bilirubin which differs much from indirect reacting bilirubin in both protein binding and solubility

Peritoneal dialysis with protein enriched dialysing fluid offers the technical possibility of extracting significant amounts of indirect reacting bilirubin from infants and newborns but dialysance is rather low A rapidly increasing jaundice cannot be checked by this method and it is advisable to use exchange transfusion for the treatment of this condition if treatment is considered necessary

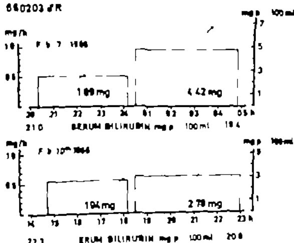


*Fig 1* Graphical presentation of bilirubin values in relation to each installation Dotted lines represent bilirubin concentration in dialysing fluid *The height* of boxes indicate bilirubin extraction per hour the area gives the extraction in mg as *stated in each* box

Table 2 *Pertinent values o e nt case and h one of Grollman & Odell (3)*

| | Present case | Grollman & Odell |
|---|---|---|
| Body weight kg | 1 6 | 65 |
| Plasma volume ml | 64 | 4 000 |
| Bilirubin space ml | 256 | 16 000 |
| Indirect reacting bilirubin in serum (mean) mg/100 ml | 21 | 16 |
| Total body bilirubin mg | 53 8 | 2 560 |
| Bilirubin extraction (mean) mg/h | 0 67 | 29 |
| Bilirubin extraction in relation to total body bilirubin | 1 2 | 1 2 |
| T/2 hrs | 57 9 | 57 9 |
| Dialysance ml/h | 3 2 | 181 |
| Duration of dialysis hrs | 16 2 | 10 |
| Total bilirubin extraction mg | 10 83 | 310 4 |
| Protein concentration in dialysing fluid g/100 ml | 5 | 4 |
| Volume of installation ml/kg | 50 | 30 |

In the last case values concerning direct reacting bilirubin have been omitted

## ACKNOWLEDGEMENT

Dr Henrik Hertz Protein Laboratory Institute of General Pathology University of Copenhagen is thanked for p rforming the bilirubin estimations

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*Key words* Hyperbilirubinaemia newborn infant peritoneal dialysis

Table 2. *Effect of pyridoxine administration on urinary cystathionine excretion in 4 children*

| Case No | Date | Pyridoxine 50 mg daily | Cystathionine in mg/g creatinine | α-amino nitrogen in mg/g creatinine | Remarks |
|---|---|---|---|---|---|
| 1 | 26/5 | no | 0 | 394 | Pyridoxine treatment from 12/7 to 26/7 |
| | 9/6 | no | 0 | 578 | |
| | 6/7 | no | 160 | 556 | |
| | 17/7 | yes | 80 | 530 | |
| | 20/7 | yes | 3 | 389 | |
| | 24/7 | yes | 0 | 1190 | |
| | 16/8 | no | 0 | 1120 | |
| | 21/11 | no | 0 | 634 | |
| 2 | 10/3 | no | 220 | 567 | Pyridoxine treatment from 29/3 to 18/4 |
| | 20/3 | no | 180 | 545 | |
| | 30/4 | yes | 4 | 329 | |
| | 10/4 | yes | 0 | 410 | |
| | 13/4 | yes | 0 | 548 | |
| | 1/6 | no | 0 | 320 | |
| | 5/12 | no | 0 | 182 | |
| 3 | 4/10 | no | 100 | 703 | Pyridoxine treatment from 29/10 to 26/11 |
| | 13/10 | no | 110 | 215 | |
| | 1/11 | yes | 0 | 268 | Convulsions disappeared during treatment |
| | 2/11 | yes | 0 | 240 | |
| | 3/11 | yes | 0 | 379 | |
| 4 | [illegible]9/10 | no | 15 | 394 | Pyridoxine treatment from 21/10 to 6/3 |
| | 3/10 | no | 10 | 300 | |
| | 19/12 | no | 35 | 405 | |
| | 1/12 | yes | 0 | 285 | Convulsions disappeared during treatment |
| | 11/1 | yes | 0 | 308 | |
| | 4/3 | yes | 0 | 185 | |

As seen in Table 1 only the children with severe liver malfunction (nos. 1 and 2) showed an increased excretion of α-amino-nitrogen during the course of the disease. In these 2 patients this increase was due to a general aminoaciduria. In the other 4 patients no change in the urinary aminoacid pattern could be seen. No changes in the taurine excretion during the pyridoxine treatment could be registered.

## DISCUSSION

None of the 6 cases of cystathioninuria detected during the screening of 230 children with various diseases can be classified as primary or congenital cystathioninuria. Two patients (nos. 1 and 2) had impaired liver function and there is no doubt that the cystathioninuria is related to their liver disease. Shaw *et al.* (11) reported cystathioninuria in several children with various disease associated with gross liver failure: untreated galactosaemia (3 cases), glycogen storage disease (1 case before death), hepatoma (3 cases), tyrosinosis (1 case) and one case of probably congenital portal vein obstruction. The occurrence of cystathionine in urine from a patient with hepatoma has been described earlier by Gjessing & Mauritzen (5).

Cystathioninuria was induced experimentally in pyridoxine-deficient rats (Blaschko *et al.* (1), Hope (7)) and is known to occur in pyridoxine-deficiency in man (3, 10). The probability of cystathioninuria being an expression of pyridoxine-deficiency in these patients with liver malfunction cannot be decided on the basis of the available data. The positive response, i.e. the disappearance of cystathioninuria on treatment with pyridoxine in both cases with gross liver malfunction speaks in favour of a deficiency-state. These patients certainly had during the course of their disease a reduced intake of food due to anorexia and vomiting. In addition one can expect an impaired handling of pyridoxine by the diseased liver.

The cystathioninuria registered in patient

Table 1 *Clinical and laboratory findings in 6 children with cystathioninuria*

| Case no | Age | Sex | Diagnosis | Cystathionine* in urine | α amino * nitrogen in urine | Symptoms at admission | Laboratory findings |
|---|---|---|---|---|---|---|---|
| 1 | 1 week | male | Severe liver mal function + Hydro nephrosis neona torum (suspect neo natal hepatitis) | 160 | 1190 | Not thriving Retentio testis Asymetrical face | Bil 7 8 mg GOT 240 GPT 179 |
| 2 | 4 weeks | female | Severe liver mal function (suspect neonatal hepatitis) | 220 | 567 | Vomiting + Icterus | Bil 7 4 mg GOT 69 GPT 78 alk phos 50 |
| 3 | 4 weeks | male | Convulsions + Encephalopathia infantilis | 110 | 329 | Convulsions | Atypical EEG changes No liver affection |
| 4 | 4 months | female | Convulsions of unknown cause | 35 | 384 | Convulsions + fever after smallpoxvacci nation | No EEG-changes No liver affection |
| 5 | 9 months | male | Epilepsy + Microcephalia + micropthalmia | 80 | 278 | Convulsions Not thriving | EEG-change typical for epilepsy No liver affection |
| 6 | 3 months | male | Severe congenital multiple malforma tions + broncho pneumonia | 60 | 180 | See diagnosis | Urine investigate 1 day before death |

* Highest registrated value during hospital stay The values are expressed in mg per g creatinine

method was about ± 20 % as checked by experiments with addition of known amounts of authentic cysta thionine and comparative studies after photometric evaluation of ninhydrin treated cystathionine spots

## RESULTS

In 6 of 230 children urinary excretion of cystathionine could be detected The urinary excretion varied between 35 and 220 mg per g of creatinine The diagnosis, past history clinical and laboratory findings of these 6 patients are given in Table 1 All 6 children were below the age of 9 months when the cystathionine was detected In 4 (case nos 1–4) of the 6 children the cystathionine excretion was measured at different time periods before during and after a treatment with 80 mg pyridoxine daily (see Table 2) In all 4 patients a decrease of the urinary excretion of cystathionine could be registered After 12 days of treatment no cystathionine could be detected in the urine (detection limit 0 2 $\mu$g per mg creatinine) In the two children (case nos 3 and 4) who had convulsions as their main symptom the convulsions disappeared during the pyridoxine treatment although this disappearence was not a sudden one Patient no 5 had also convul sions was not treated with pyridoxine during the cystathionine study and was diagnosed as having epilepsy and had microcephalia and micropthalmia This patient was later on treated with pyridoxine without effect on the convulsions Patient no 6 was born with mul tiple severe malformations and the cystathioninuria was found one day before the child died in respiratory insufficiency

Patient no 1 and no 2 were 7 weeks and 4 weeks, respectively when the cystathioninuria was detected Both had hyperbilirubinaemia (direct reacting) increases in their serum trans aminase activities and urinary excretion of bile pigments In both children a severe hepatic malfunction was found No biopsy was per formed and a final diagnosis was not made No signs of liver involvement could be registered in the other 4 cases (nos 3–6) with cystathioninuria

Table 2 *Effect of pyridoxine administration on urinary cystathionine excretion in 4 children*

| Case No | Date | Pyridoxine 80 mg daily | Cystathionine in mg/g creatinine | α amino nitrogen in mg/g creatinine | Remarks |
|---|---|---|---|---|---|
| 1 | 26/5 | no | 0 | 394 | Pyridoxine treatment from 12/7 to 26/7 |
| | 9/6 | no | 0 | 578 | |
| | 6/7 | no | 160 | 556 | |
| | 17/7 | yes | 80 | 530 | |
| | 20/7 | yes | 3 | 589 | |
| | 24/7 | yes | 0 | 1190 | |
| | 16/8 | no | 0 | 1120 | |
| | 21/11 | no | 0 | 636 | |
| 2 | 10/3 | no | 220 | 567 | Pyridoxine treatment from 29/3 to 11/4 |
| | 0/3 | no | 160 | 545 | |
| | 30/4 | yes | 4 | 549 | |
| | 10/4 | yes | 0 | 410 | |
| | 13/4 | yes | 0 | 548 | |
| | 21/4 | no | 0 | 370 | |
| | 5/12 | no | 0 | 382 | |
| 3 | 4/10 | no | 100 | 303 | Pyridoxine treatment from 29/10 to 26/11 |
| | 13/10 | no | 110 | 215 | |
| | 1/11 | yes | 0 | 268 | Convulsions disappeared during treatment |
| | 2/11 | yes | 0 | 240 | |
| | 3/11 | yes | 0 | 329 | |
| 4 | 29/10 | no | 35 | 384 | Pyridoxine treatment from 21/10 to 6/3 |
| | 25/10 | no | 30 | 300 | |
| | 19/12 | no | 35 | 405 | |
| | 21/12 | yes | 0 | 285 | Convulsions disappeared during treatment |
| | 11/1 | yes | 0 | 308 | |
| | 6/3 | yes | 0 | 183 | |

As seen in Table 1 only the children with severe liver malfunction (nos 1 and 2) showed an increased excretion of α-amino-nitrogen during the course of the disease. *In these 2* patients this increase was due to a general aminoaciduria. In the other 4 patients no change in the urinary aminoacid pattern could be seen. No changes in the taurine excretion during the pyridoxine treatment could be registered.

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The cystathioninuria registered in patient

Table 1. *Clinical and laboratory findings in 6 children with cystathioninuria*

| Case no. | Age | Sex | Diagnosis | Cystathionine* in urine | α-amino-* nitrogen in urine | *Symptoms* at admission | *Laboratory* findings |
|---|---|---|---|---|---|---|---|
| 1 | 1 week | male | Severe liver malfunction + Hydronephrosis neonatorum (suspect neonatal hepatitis) | 160 | 1190 | Not thriving Retentio testis Assymetrical face | Bil 7.8 mg GOT 240 GPT 179 |
| 2 | 4 weeks | female | Severe liver malfunction (suspect neonatal hepatitis) | 220 | 567 | Vomiting + Icterus | Bil 7.4 mg GOT 69 GPT 78 alk. phos. 50 |
| 3 | 4 weeks | male | Convulsions + Encephalopathia infantilis | 110 | 329 | Convulsions | Atypical EEG changes. No liver affection |
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| 5 | 9 months | male | Epilepsy + Microcephalia + micropthalmia | 80 | 278 | Convulsions Not thriving | EEG-change typical for epilepsy. No liver affection |
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Highest registrated value during hospital stay. The values are expressed in mg per g creatinine.

method was about ± 20% as checked by experiments with addition of known amounts of authentic cystathionine and comparative studies after photometric evaluation of ninhydrin treated cystathionine spots.

## RESULTS

In 6 of 230 children urinary excretion of cystathionine could be detected. The urinary excretion varied between 35 and 220 mg per g of creatinine. The diagnosis, past history, clinical and laboratory findings of these 6 patients are given in Table 1. All 6 children were below the age of 9 months when the cystathionine was detected. In 4 (case nos. 1–4) of the 6 children the cystathionine excretion was measured at different time periods before, during and after a treatment with 80 mg pyridoxine daily (see Table 2). In all 4 patients a decrease of the urinary excretion of cystathionine could be registered. After 12 days of treatment no cystathionine could be detected in the urine (detection limit 0.2 μg per mg creatinine). In the two children (case nos. 3 and 4) who had convulsions as their main symptom, the convulsions disappeared during the pyridoxine treatment although this disappearence was not a sudden one. Patient no. 5 had also convulsions, was not treated with pyridoxine during the cystathionine study, and was diagnosed as having epilepsy and had microcephalia and micropthalmia. This patient was later on treated with pyridoxine without effect on the convulsions. Patient no. 6 was born with multiple severe malformations and the cystathioninuria was found one day before the child died in respiratory insufficiency.

Patient no. 1 and no. 2 were 7 weeks and 4 weeks, respectively when the cystathioninuria was detected. Both had hyperbilirubinaemia (direct reacting), increases in their serum transaminase activities and urinary excretion of bile pigments. In both children a severe hepatic malfunction was found. No biopsy was performed and a final diagnosis was not made. No signs of liver involvement could be registered in the other 4 cases (nos. 3–6) with cystathioninuria.

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12 Smith, I *Chromatographic and Electrophoretic Techniques* William Heinemann Medical Books Ltd. London 1960 Vol 1 pp 95 98

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no. 6 may be interpreted as an "obvious fore-runner" of death in the same way as Shaw and coworkers (11) described this phenomenon in one patient with maple syrup disease and one with glycogen storage disease in the final stage. Here the cystathioninuria is obviously due to derangement of the liver function.

*It is more difficult to explain the three re*maining cases of cystathioninuria (nos. 3, 4 and 5). All three had been admitted to hospital for convulsions. They had no signs of liver disease. Two of them (nos. 3 and 4) were *treated with pyridoxine and the cystathioninu*ria disappeared as well as the convulsions. One child (no. 5) received later on pyridoxine without any effect on its convulsions. No amino acid study was done during this treatment. The disappearance of the convulsions in patient nos. 3 and 4 was not a dramatic one as reported for patients with pyridoxine dependency. No reappearance of convulsions or cystathioninuria was seen after withdrawal of pyridoxine. In addition cystathioninuria does not belong to the dependency syndrome either. On the other hand several reports are known (for *review see Hansson & Hagberg (6)) where chil*dren with convulsions responded well to pyridoxine treatment. These reports do not mention anything about cystathioninuria except in one report in which the 14 month old child was classified as "vitamin $B_6$ deficient" (10). In this case the seizures and cystathioninuria disappeared after pyridoxine treatment. The possi*bility could be discussed if patient nos.* 3 and 4 belong to the same group of children for whom Hansson & Hagberg (6) proposed the term "pyridoxine-responsive seizures". However, the clinical picture in the here reported cases was very heterogenous and differed in most respects from those described by Hansson & Hagberg (6). Since there is the report of Perry *et al.* (9) of two healthy siblings with congenital cystathioninuria the possibility must be considered that the cystathioninuria in some cases described here is only coincidental and in no way related to the disease. Perry and coworkers (9) also registered a reduction of urinary cystathionine after pyridoxine treatment in their cases. Further serial studies in larger groups of hospitalized and healthy children would help clarify this question.

## SUMMARY

Cystathioninuria was *detected in 6 of 230 hos*pitalized children. Three had convulsions when admitted to the hospital, two severe liver malfunction of unknown cause (suspect neonatal hepatitis) and one child had severe congenital malformations and bronchopneumonia and *was in a terminal stage. Cystathioninuria dis*appeared in 4 children (two with convulsions *and two with liver malfunction) treated with* pyridoxine orally. Pyridoxine treatment (2×40 mg daily) also resulted in disappearence of convulsions. The possible background to these findings is discussed.

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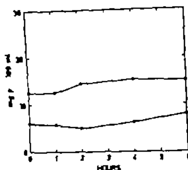



Fig 1 Case E B Serum concentrations of methylmalonate during an oral tolerance test with L valine in a dose of 100 mg per kg body weight (—•) and during a repeated test one hour after the intramuscular administration of 5 mg of cobamide coenzyme (o—o)

During the period of study both patients were on a fixed diet providing them with 2.0 g of protein per kg per day In each of them two oral tolerance tests with L valine a precursor of methylmalonic acid were performed with an interval of 48 hours The L valine was dissolved in water and was given in a dose of 100 mg per kg body weight at 9 a m when the patients were fasting During the subsequent 6 hours only fruit juice was given Venous blood samples were drawn from an indwelling catheter at zero time and 1 2 4 and 6 hours after the test dose had been given Serum was kept frozen until analysed One hour before the second tolerance test with L valine 5 mg of 5 6-dimethylbenzimidazole cobamide coenzyme (kindly supplied by Glaxo Laboratories Ltd Greenford, Middlesex England) was given intramuscularly The cobamide coenzyme was dissolved in 2 3 ml of a 0.9 per cent sodium chloride solution Urine was collected in 2 to 24 hour periods for 4

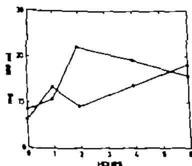



Fig 2 Case S B Serum concentrations of methylmalonate during an oral tolerance test with L valine in a dose of 100 mg per kg body weight (—•) and during a repeated test one hour after the intramuscular administration of 5 mg of cobamide coenzyme

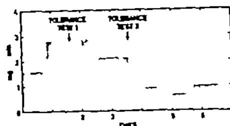



Fig 3 Case E B Rate of urinary excretion of methylmalonic acid During the period of study two peroral tolerance tests with L valine in a dose of 100 mg per kg body weight were performed Cobamide coenzyme in a dose of 5 mg was given intramuscularly one hour before the second test The body weight of the patient was 14.5 kg

and 7 days respectively and kept in a deep freeze until analysed Methylmalonic acid in serum and in urine was determined as described by Oberholzer et al (5)

The serum concentrations of methylmalonate during the two tolerance tests with L valine are shown in Figs 1 and 2 The final increase in the concentration of methylmalonate during the whole 6 hour period was about the same in both instances in the two patients However the initial increase was slower after the administration of cobamide coenzyme

The rates of urinary excretion of methyl

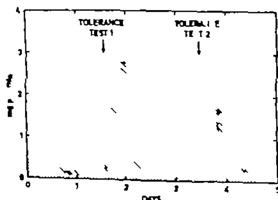



Fig 4 Case S B Rate of urinary excretion of methylmalonic acid During the period of study two peroral tolerance tests with L valine in a dose of 100 mg per kg body weight were performed Cobamide coenzyme in a dose of 5 mg was given intramuscularly one hour before the second test The body weight of the patient was 12.9 kg

SHORT COMMUNICATION

# THE EFFECT OF COBAMIDE COENZYME IN METHYLMALONIC ACIDEMIA

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*From the Department of Physiological Chemistry Chemical Centre University of Lund Lund and the Department of Pediatrics at Crown Princess Louisa s Children s Hospital Karolinska Institutet Stockholm Sweden*

Methylmalonic acidemia (8) or aciduria (5) is a newly described inborn error of metabolism Clinically the disease is characterized by metabolic acidosis, vomiting dehydration, muscular hypotonia, and retarded psychomotor development Life threatening crises of acidosis may develop during the course of even mild acute infections or during periods of high protein intake (3) The main biochemical features are methylmalonic acidemia high urinary excretion of methylmalonic acid and intermittent ketosis

The accumulation of methylmalonic acid is caused by a block in the two step conversion of methylmalonyl CoA to succinyl CoA (4 6 7) In the first step the enzyme methylmalonyl CoA racemase (EC 5 1 99 1) catalyzes the reversible conversion of D methylmalonyl CoA to L-methylmalonyl CoA and in the second step methylmalonyl CoA mutase (EC 5 4 99 2) catalyzes the reversible conversion of L methylmalonyl CoA to succinyl CoA The last enzyme has cobamide coenzyme as a co factor

The metabolic block in methylmalonic acidemia may thus be explained by a deficiency of one or both of the two enzymes Since patients with pernicious anemia are known to excrete up to 1 g of methylmalonic acid in the urine per day (1 2) the possibility exists that there is in methylmalonic aciduria, an abnormally high requirement of cobamide coenzyme i e a so called dependency condition In order to test the validity of this hypothesis, the metabolic effect of a large dose of cobamide coenzyme has been studied in two patients with methylmalonic acidemia

The first patient (case E B) is a girl of 2 9/12 years of age and whose clinical history has been reported in detail previously (3) The second patient (case S B) is a 2 year old boy with a history of mild to moderate attacks of metabolic acidosis since the neonatal period When 1 7/12 years of age he got an acute upper respiratory tract infection and during the course of this disease he developed a severe ketosis and a pronounced metabolic acidosis and went into shock Following intensive medical treatment including the administration of large doses of sodium bicarbonate he recovered from the acute episode However when the protein intake was increased after another week the patient relapsed Since it was then found that the urinary excretion of methylmalonic acid ranged between 3 and 4 g per day and that the serum concentration of this compound was 23 mg per 100 ml he was diagnosed as a case of methylmalonic acidemia When the patient had recovered from the second severe attack of acidosis he was put on a low protein diet providing him with 2 g of protein per kg body weight per day Since then he has been free of severe attacks of acidosis although he has continued to excrete large quantities of ketone bodies in the urine There has never been any symptoms of vitamin $B_1$ deficiency and the serum concentration of vitamin B has been found to be 690 pg per ml

This work has been supported by a grant from the Swedish Medical Research Council (2583)

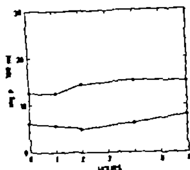


Fig 1 Case E B Serum concentrations of methylmalonate during an oral tolerance test with L valine in a dose of 100 mg per kg body weight (•—•) and during a repeated test one hour after the intramuscular administration of 5 mg of cobamide coenzyme (○—○)

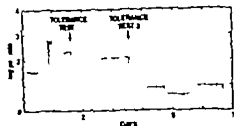



Fig 3 Case E B Rate of urinary excretion of methylmalonic acid During the period of study two peroral tolerance tests with L valine in a dose of 100 mg per kg body weight were performed Cobamide coenzyme in a dose of 5 mg was given intramuscularly one hour before the second test The body weight of the patient was 14 5 kg

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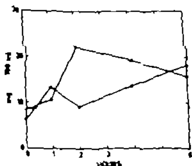



Fig 2 Case S B Serum concentrations of methylmalonate during an oral tolerance test with L valine in a dose of 100 mg per kg body weight (•—•) and during a repeated test one hour after the intramuscular administration of 5 mg of cobamide coenzyme

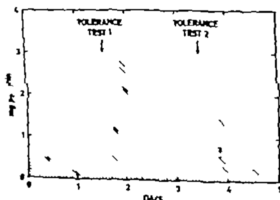



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The accumulation of methylmalonic acid is caused by a block in the two step conversion of methylmalonyl CoA to succinyl CoA (4 6 7) In the first step the enzyme methylmalonyl CoA racemase (EC 5 1 99 1) catalyzes the reversible conversion of D methylmalonyl CoA to L-methylmalonyl CoA and in the second step methylmalonyl CoA mutase (EC 5 4 99 2) catalyzes the reversible conversion of L methylmalonyl CoA to succinyl CoA The last en zyme has cobamide coenzyme as a co factor

The metabolic block in methylmalonic acidemia may thus be explained by a defi ciency of one or both of the two enzymes Since patients with pernicious anemia are known to excrete up to 1 g of methylmalonic acid in the urine per day (1 2) the possibility exists that there is in methylmalonic acidemia, an abnormally high requirement of cobamide coenzyme i e a so called dependency condi tion In order to test the validity of this hypo thesis the metabolic effect of a large dose of cobamide coenzyme has been studied in two patients with methylmalonic acidemia

The first patient (case E B) is a girl of 7 9/12 years of age and whose clinical history has been re ported in detail previously (3) The second patient (case S B) is a 2 year-old boy with a history of mild to moderate attacks of metabolic acidosis since the neonatal period When 1 7/12 years of age he got an acute upper respiratory tract infection and during the course of this disease he developed a severe ketosis and a pronounced metabolic acidosis and went into shock Following intensive medical treatment in cluding the administration of large doses of sodium bicarbonate he recovered from the acute episode However when the protein intake was increased after another week the patient relapsed Since it was then found that the urinary excretion of methylmalonic acid ranged between 3 and 4 g per day and that the serum concentration of this compound was 23 mg per 100 ml he was diagnosed as a case of methylmalonic acidemia When the patient had recovered from the second severe attack of acidosis he was put on a low protein diet providing him with 2 g of protein per kg body weight per day Since then he has been free of severe attacks of acidosis although he has continued to excrete large quantities of ketone bodies in the urine There has never been any symptoms of vitamin $B_{12}$ deficiency and the serum concentration of vitamin $B_{12}$ has been found to be 690 pg per ml

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This work has been supported by a grant from the Swedish Medical Research Council (2583)

*Acta Pædiat Scand 58* 181–186 1969

## CASE REPORT

# AN INHERITED B/C TRANSLOCATION IN A DYSPLASTIC GIRL WITH PARTIAL C TRISOMY

BERTIL HALL and NILS SVENNINGSEN

*From the Institute of Genetics and the Department of Pediatrics University of Lund Lund Sweden*

Reciprocal translocations are now well documented in man The present report describes a new instance of a reciprocal translocation between a B and a C chromosome Other reciprocal translocations between these two chromosome types have been reported before

## CASE REPORT

The patient is an infant girl second born child of a 21 year-old mother and 29 year-old father The first born sibling is healthy There is no family history of malformations or consanguinity

The infant was born in the 40th week of gestational age and had a birth weight of 2290 g The placental weight was 410 g Because of peculiar external abnormalities a closer examination including a chromosomal examination was performed

### *Physical examination*

Physical examination showed a newborn rather pale infant with slight muscular hypotonia and no Moro reflex response A certain jitteriness was observed during the neonatal period The cry was feeble and high pitched The head was brachy and microcephalic (head circumference 30 cm) with a flat occiput The skin was generally loose The slant of the palpebral fissures was normal (Fig 1 *a*) but a slight sunset phenomenon was constantly observed There was a pronounced micrognathia (Fig 1 *b*) The ears were low set and slightly dysplastic with deep conchae large antihelix and antitragus

There was a hyperextensibility in the radiocarpal joints and in the proximal finger joints giving the fingers a *swan neck-like* appearance The fingers were slender and long and the nails were hyperconvex A simian crease was observed in the right hand and a partial simian crease in the left hand The dermatoglyphic pattern showed six arches on the finger tips The ATD angle was normal A moderate *pes excavatus* was found

### *Laboratory data*

Routine blood examinations showed no abnormalities The trombocyte and white blood cell counts were normal Bone marrow examination was normal Serum electrolytes serum protein electrophoresis urinary amino acids blood urea were all normal EEG at 3 weeks of age showed abnormally slow activity bitemporally but it seemed to be normal when repeated at 2 months of age

Leucocyte alkaline phosphatase was low (alk phosph score = 12) at 2 months of age but was normal (alk phosph score = 107.5) at 3 months of age Erythrocyte glucose 6-phosphate dehydrogenase showed a low activity (96 u/100 ml E) and pyruvatkinase was normal (417 u/100 ml E) Galactose 1 phosphate uridyl transferase activity was 39.4 u/g Hb which is abnormal Fetal hemoglobin was 3.5 at 17 months of age

X ray examination of the pelvis and thorax skeleton showed no abnormalities and the bone age was normal X ray of hands showed dysplastic middle phalanges of the fifth fingers and dysplastic distal phalanges of all fingers (Fig 2)

The blood groups were the expected according to the blood types of the parents No incompatibility was found

### *Progress*

During the first month of life the infant had extreme muscular hypotonia with a paucity of motility but after 1 month of age the muscular tone was increased and the initial signs of "failure to thrive" slowly subsided and at 3 months of age she could be discharged from the hospital At the age of 17 months she was reexamined She had a retarded psychomotor development not being able to sit upright by herself

malonic acid during the period of study are shown in Figs 3 and 4 After the first tolerance tests the rates of excretion increased steadily in case E B for a period of 21 hours and in case S B for 11 hours When the tolerance tests were repeated one hour after the administration of cobamide coenzyme the increase in the rates of excretion was much less in both patients In case E B the rate of excretion was even below the basal value i e the value found before the first test The total amount of methylmalonic acid excreted by case E B during the first 24 hours following the tolerance test when the cobamide coenzyme had been given was only 1 65 g compared to at least 3 43 g during the same period after the first tolerance test when the cobamide coenzyme had not been given Because a portion of the urine during the first tolerance test was lost only a minimum value can be given In case S B the corresponding values were 0 69 g and 1 44 g respectively In this connection it is interesting to note that patient S B who during a previous 6 week period on the same protein intake, almost constantly had been found to have a high urinary excretion of ketone bodies, as revealed from strongly positive Ketostix® tests had no positive test for a period of one month after the administration of a single dose of cobamide coenzyme

From the results obtained it is evident that the administration of a large dose of cobamide coenzyme has an effect on the metabolism of methylmalonic acid in cases of methylmalonic acidemia The results also strongly suggest that the metabolic defect is localized to the reaction L methylmalonyl CoA ⇌ succinyl CoA However specific enzymatic studies are required to determine if the deficiency of the holoenzyme methylmalonyl CoA mutase is caused by a deficiency of the apoenzyme or by an abnormality in the interaction between the apoenzyme and the coenzyme Although there is no vitamin $B_{12}$ deficiency the deficiency could also be caused by an inadequate coenzyme pool

The therapeutic implications of the findings are that large doses of cobamide coenzyme might be beneficial during crises of acidosis in patients with methylmalonic acidemia

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*Note added in proof*

Since this paper was submitted Rosenberg L E Lilljequist A C and Hsia Y E (New Eng J Med *278* 1319 1968) have reported a marked decrease of the urinary excretion of methylmalonic acid after the administration of large doses of vitamin B to a patient with methylmalonic acidemia


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*Key words* Cobamide coenzyme methylmalonic acidemia methylmalonic aciduria tolerance test vitamin dependency syndrome

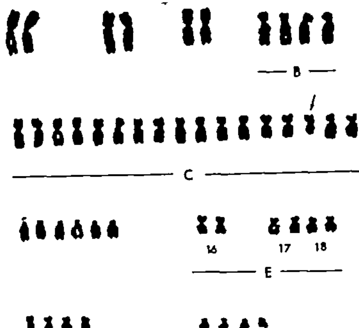



*Fig 4* Karyotype of the proposita with 47 chromosomes The extra chromosome (*arrow*) cannot on morphological grounds be separated from the chromosomes in pair No 16 This chromosome should however be identical with the deleted C (No 11) chromosome found in the karyotype of the mother which is why it is placed in the C group Note that the rest of the chromosomes have normal morphology

The other 46 chromosomes had a normal morphology The father had normal chromosomes The mother and the sister of the proposita were both normal phenotypically but showed a structural chromosomal aberration The modal chromosome number was 46 One C chromosome was replaced by a chromosome with the same morphology as a chromosome of pair 16 and in addition the long arm of one of the B chromosomes was longer than usual (Fig 6) The most probable interpretation of the chromosome findings in the mother and her phenotypically normal daughter is that there has been a reciprocal interchange of unequal portions of arms between one of the group C and one of the group B chromosomes with the result that a portion of the long arm of a C chromosome has become attached to the long arm of a B chromosome (Fig 7) When pairing the chromosomes in the C group there were indications that the deleted C chromosome belonged to pair No 11 (Fig 6) The proposita thus is a partial C trisomy and probably has a deficiency for a small part of a B chromosome and C chromosome

Examination of buccal smears of the proposita showed normal positiv sex chromatin

## DISCUSSION

There are other partial C trisomies reported (see Table 1) The phenotypes of the present patient and those earlier reported are not similar Another partial C trisomy investigated in this laboratory (Haeffler & Hall) also showed quite a dissimilar phenotype with the proposita The present patient shows some signs also encountered in other chromosomal syndromes like the abnormal cry the absence of the Moro reflex brachycephaly loose skin micrognathia dysplastic ears simian crease six arches on the finger tips deviating glucose-6-phosphate de







Fig 1 A face and profile photograph of the proposita Note the pronounced microgenathia

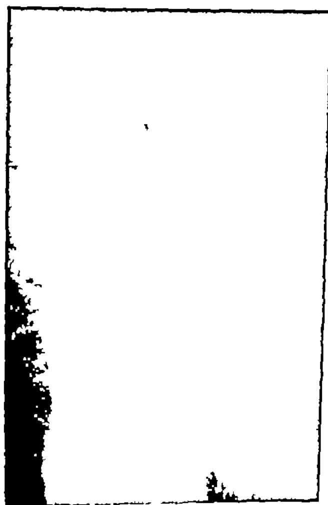


Fig 2 X ray picture of the hand of the proposita Note the dysplastic middle phalanx of the little finger and the dysplastic distal phalanges

A mutual contact has been established between the mother and her child and the child is even able to respond by smiling Besides a few upper respiratory infections and urinary infections she has been well However the weight gain had been poor especially after one year of age the weight at 17 months of age was 6900 g

An urography at the age of 30 months showed broad and coarse calices

*Cytogenetic investigation*

The chromosomes of the patient, her sister and parents were studied in blood cultures (Fig 3) The maternal grandparents are still living but were not available for chromosomal studies The proposita had 47 chromosomes in all cells studied (Fig 4) The extra chromosome could not be separated on morphological grounds from the chromosomes of pair No 16 (Fig 5)

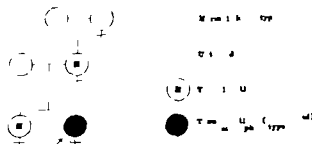


Fig 3 This pedigree shows the inheritance of the translocation

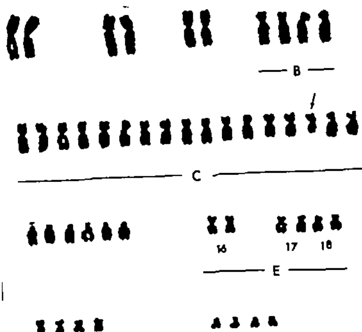


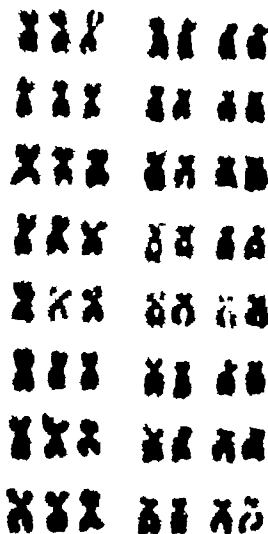

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hydrogenase activity (14) and high activity of the enzyme galactose 1-phosphate uridyl transferase (10)

According to earlier reports six arches a characteristic sign of the E trisomy syndrome is not quite unusual in patients with other chromosomal aberrations (9 15) and in persons with normal chromosomes (2) Of the partial C trisomies referred to in this paper 6/13 have 5 or more arches (the dermatoglyphs of six of the patients are not presented) (see Table 1) one has 5 arches (4) three have six arches (7 15) and one has nine arches (11) It must be pointed out that it is not certain that these patients are pure, partial C trisomies There is also the possibility of a small deficiency for a part of the chromosomes implicated in the translocation

The overlapping of signs between different chromosome syndromes are in accordance with

*Fig 5* This picture shows the impossibility of separating the small translocation chromosome from chromosomes of pair No 16 To the right in the picture are chromosome pairs Nos 17 and 18 from the corresponding metaphase plates

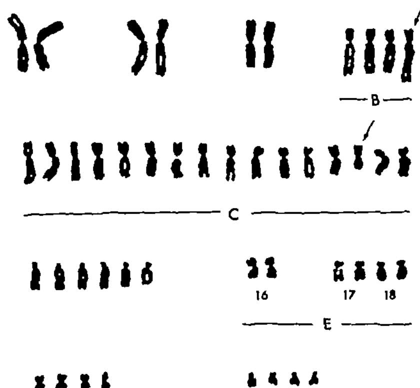



*Fig 6* Karyotype of the mother with 46 chromosomes and two translocation chromosomes (*arrows*) B/C translocation The sister of the proposita has a morphologically identical karyotype

Table 1 Partial C trisomies

The table shows from which translocation the partial C trisomies originate + Means that the patient has 5 or more arches on the fingertips and ? means that the dermatoglyphs have not been presented

| | A/C | B/C | C/C | D/C | E/C | F/C | G/C |
|---|---|---|---|---|---|---|---|
| Edwards *et al* (1962 | | – | | | | | |
| Edwards *et al* (1962) | | – | | | | | |
| Rohde & Catz (1964) | | | ? | | | | |
| de Grouchy & Canet (1965) | | | | + | | | |
| Lindsten *et al* 1965) | | | + | | | | ? |
| Giray *et al* (1966) | | | | | | | ? |
| Giray *et al* (1966) | | | | | | | |
| Kaplan *et al* (1966) | | | | | | + | |
| Punnett *et al* (1966) | | | | | + | | |
| Buhler *et al* (1967) | | | | | – | | |
| de Grouchy *et al* (1967 *a*) | | – | | | | | |
| de Grouchy *et al* (1967 *b*) | | | | – | | | |
| Jensen & Melchior (1967) | | | | ? | | | |
| Lord *et al* (1967) | | | | | | | |
| Lord *et al* (1967) | | | | | | | – |
| McKusick *et al* (1967) | | | | | | | |
| Mikkelsen *et al* (1968) | | | | | | | ? |
| Present case | | + | | | | | |
| Haeffler & Hall (Unpublished) | | | + | | | | |
| | 0 | 5 | 3 | 3 | 2 | 1 | 5 |

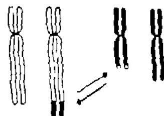


Fig 7 This is a schematic drawing of the two pairs of chromosomes involved in the translocation one pair of the B chromosomes and one pair of the C chromosomes (probably No 11)

some earlier reports (3 8 15) The pronounced micrognathia of the present patient gave her however a characteristic facial appearance

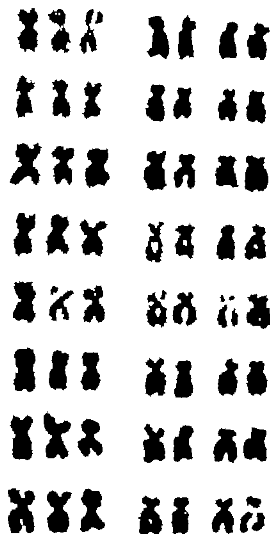
The sister with a balanced translocation should in our opinion be advised in due time not to bear any children because of the high risk of having chromosomally abnormal and dysplastic children as is clarified by earlier studies and the present investigation

## SUMMARY

A new case of partial C trisomy with many signs encountered in other chromosomal syndromes is reported The dermatoglyphic findings on the finger tips are discussed

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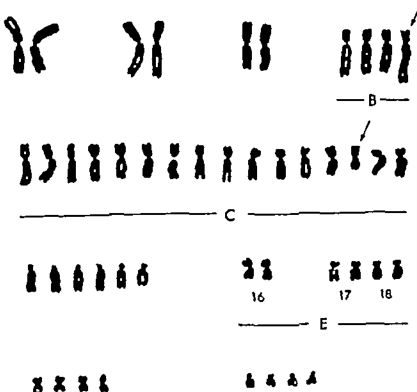



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CASE REPORT

# NEONATAL NONSPHEROCYTIC HEMOLYTIC ANEMIA DUE TO GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN A DANISH INFANT

J E OLSEN

*From the University Department of Pediatrics (Head Torben Iversen) Aarhus Kommunehospital Aarhus Denmark*

Nonspherocytic hemolytic anemia of the new born may be caused by congenital enzymatic defects of the intermediary metabolism of the red blood cells Glucose 6 phosphate dehydrogenase (G 6-PD) deficiency is by far the most frequent of these disorders In the following is described an infant with this abnormality which has not previously been reported from Denmark

## CASE REPORT

The patient is a boy who is the second of two siblings. He has a four year old sister who is healthy So are both his parents who are of Danish descent and not consanguineous There is no disposition for blood diseases or jaundice in the family

The pregnancy was uncomplicated Five days before delivery the mother got 20 mg of menadione sodium bisulfite otherwise she received no medicine Vitamin K had not been administered to either mother or child in direct relation to delivery The delivery took place at the local hospital at the expected time without any complications The birth weight was 2800 g and the length 51 cm

One hour after being born the patient developed icterus. The blood type of both mother and child was O Rh D positive Immediately thereafter the patient was transferred to the Obstetrical Department of the University of Aarhus When admitted there ten hours after delivery the patient was moderately icteric but otherwise normal The total serum bilirubin value was 7 8 mg/100 ml all of which was of the indirect type On the fourth day a maximum value of 18 4 mg/100 ml was reached of which the direct bilirubin fraction amounted 0 2 mg/100 ml On the second day of life the patient's hemoglobin (Hb) concentration was 100 % by the Sahli method Coombs test and dextrane reaction were negative

During admission the patient showed no signs of bleeding or infection, the jaundice diminished and nine days old he was discharged to his home where the icterus vanished completely

One month old he was re admitted to the local hospital because of bad thriving On admission he was found extremely emaciated non icteric The Hb concentration was 5 2 g/100 ml, MCV 108 millipicoliter MCHC 31 g/100 ml the reticulocyte count was 14–22 % Coombs test was negative haptoglobin 24 mg/100 ml icterus index 8 The leucocyte count was 11 400/mm with a normal differential count Examination of the bone marrow showed a considerably accentuated normoblastic erythroblastosis without side shifting There were normal amounts of blood platelets and megakaryocytes and no abnormal cell forms

At the age of 2 / months the patient was transferred to the Pediatric Department, Aarhus Kommunehospital On admission the patient was pale thin, not icteric His liver was palpable 1 /₂–2 cm below the costal margin in the medioclavicular line and the lower pole of the spleen reached /₂–1 cm below the costal margin

Laboratory findings The Hb concentration was 6 8 g/100 ml the erythrocyte count $2\,1 \times 10^6$/mm hematocrit 23 % MCV 110 millipicoliter MCHC 29 5 g/100 ml the reticulocyte count 14–24 % The leucocyte count was 8 900–10 800/mm the differential count showed 31 % neutrophile polynucleated cells and 69 % lymfocytes The thrombocyte count 560 000/mm Micro ESR 14 mm/h Haptoglobin 112 mg/100 ml Serum total bilirubin zero The urine did not contain urobilin bilirubin protein glucose or phenylpyruvic acid The sediment was normal X ray examination of the skeleton was normal

The suspicion arose that the patient was suffering from a nonspherocytic hemolytic anemia and to further elucidate this question the following tests were carried out

The osmotic fragility measured by a modified Parpart method (11) was normal After incubation for 24 hours at 37 C the osmotic fragility was increased by normal amount

Electrophoresis of hemoglobin on starch gel at a pH of 8 6 was normal

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*Key words* Chromosome aberration translocation dermatoglyphi

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The concentration of adenosinetriphosphate as sayed by an enzymatic method elaborated by Boehringer (3) was 83 mg/100 ml erythrocytes (normal value 45–65 mg/100 ml erythrocytes)

The activity of pyruvatekinase of the red blood cells by the method of Bucher & Pfleiderer (4) was 14 1 micromol/minute × g Hb – 524 micromol/min × 10 erythrocytes (normal value 7–14 micromol/minute × g Hb – 250–500 micromol/minute × 10 [*] erythrocytes)

The concentration of reduced glutathione by the DTNB method according to Beutler (1) was 76 mg/100 ml erythrocytes (normal value 65 ± 15 mg/100 ml erythrocytes) After incubation with menadione the concentration was reduced to 30 mg/100 ml erythrocytes (normal value 60 ± 15 mg/100 ml erythrocytes) This indicated a deficiency of either G 6 PD glutathione reductase or glutathione synthease

The activity of G 6 PD of the red blood cells by the method of Kornberg & Horecker (10) was in two different blood samples 0 5 and 0 72 micromol/minute × g Hb – 21 3 and 24 0 micromol/minute × $10^{10}$ erythrocytes (normal value 6–10 micromol/minute × g Hb – 150–300 micromol/minute × 10 erythrocytes) A third blood sample was examined at Rigs hospitalet Copenhagen in this sample no activity of G 6 PD at all was found (Dr Th Laursen)

Investigations carried out on blood from the patient s parents and sister yielded normal results

During hospitalization the patient has thrived well There has been a continuous reticulocytosis the Hb concentration has remained unchanged He has been seen as an out patient 5 and 9 months old At home the patient had thrived well had not been icteric His Hb concentration had risen to 9 2 g/100 ml at the latest control

## DISCUSSION

The patient developed icterus few hours after delivery and anemia was apparent already on the second day of life While the jaundice disappeared within a few weeks the anemia increased At the age of one month and again at two and a half months examination of the patients blood showed a severe normocytic anemia and a persistent reticulocytosis The bone marrow showed a picture characteristic for hemolytic anemia The spleen was slightly enlarged

Thus the patient presented signs of hemolytic anemia Isoimmunisation and infection could be excluded and toxic drugs had not been administered to the patient Examination of peripheral blood smears and bone marrow had not shown any abnormal cell forms neither in the red nor in the white blood picture

Herafter it seemed possible that the patients hemolytic anemia might be of the non spherocytic type and this suspicion was confirmed as the activity of G 6 PD was found to be markedly reduced The normal osmotic fragility of the red blood cells is in accordance with this diagnosis Curious it is, however, that the concentration of haptoglobin was normal

The frequency of G 6 PD deficiency varies greatly in different ethnic groups More than a hundred million people are assumed to have the defect Most frequently it is found among Kurds of which about 60 % are affected Rarest is G 6-PD deficiency among nonmediterranean Caucasians in whom the frequency is less than 1 %

From the Nordic countries only a few cases of G 6-PD deficiency have been published Nordøy et al (12) described two cases from Norway in a 37 year old man and his 70-year old mother From Finland Furuhjelm & Vuopio (9) briefly have published three patients whose ages were not indicated They were found amongst two families Engleson & Kjellman (8) from Sweden have examined 78 newborns with hyperbilirubinemia of unknown etiology with serum bilirubin values above 15 mg/100 ml These cases were encountered in a consecutive series of some 2500 newborns No certain case with deficient G 6 PD activity was found

The incidence of severe neonatal icterus in G 6 PD deficient subjects varies in different ethnic groups The reason for this cannot be explained on the basis of the different known types of G 6 PD deficiency

G 6 PD deficiency rarely causes neonatal jaundice in American Negroes, but is a main cause of this symptom in Greece and Sardinia However neonatal icterus is seldom due to G 6-PD deficiency in Sephardic Jews although the enzyme activity is the same in carriers of G 6 PD deficiency all over the Mediterranean area Doxiadis has proposed that another and unknown genetic factor should be responsible for the emergence of neonatal icterus in G 6 PD deficient subjects (7)

In our patient jaundice was observed already one hour after birth This seems to be very early Among Greek infants with G 6 PD deficiency Doxiadis found that icterus most frequently occurred on the second day of life

It must be considered an open question whether it is of any importance that the mother got 20 mg menadione sodium bisulfit five days before delivery In Greek infants jaundice has appeared after administration of from two to ten mg menadione bisulfit to the child in relation to delivery On the other hand icterus occurred without any known exogenuous influence in far more cases (6)

In G-6 PD deficient subjects hemolysis can be precipitated by infections and a number of different substances Besides antimalarials such as primaquine the same effect has been found of analgetics such as acetanilid acetylsalicylic acid and acetophenetidin (phenacetin) Furthermore of sulfonamides like sulfanilamide sulfapyridine sulfacetamide sulfisoxazole and of sulfones Also of furazolidone nitrofurantoin chloramphenicol and para aminosalicylic acid Moreover of naphthalene menadione sodium bisulfit (water soluble vitamin K) methylene blue phenylhydrazine quinine and quinidine (2)

G 6 PD deficiency is an inherited abnormality linked to the X chromosome It occurs homozygotic and heterozygotic in females and heterozygotic in males The lack of a family history in our case is however not astonishing, as according to Dacie (5) both parents are often found normal even when several siblings are affected

During observation the patient's concentration of Hb rose to about 9 g/100 ml Exchange transfusion or blood transfusions have not been necessary The patient must be guarded against any of the substances able to cause further hemolysis and observed carefully during infections when augmented hemolysis also may be feared

## SUMMARY


A description is given of a case of neonatal icterus and hemolytic anemia in a Danish boy in whose blood the activity of G 6 PD was markedly reduced Icterus appeared already one hour after delivery and serum bilirubin reached a maximum value of 16 4 mg/100 ml on the fourth day of life Anemia was present from the second day of life and progressed during the first month to a minimal concentration of Hb of 5 2 g/100 ml Normal activities were found in his parents and sister


## ACKNOWLEDGEMENT


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# PROCEEDINGS OF PEDIATRIC SOCIETIES

## THE EUROPEAN SOCIETY FOR PAEDIATRIC ENDOCRINOLOGY

*Abstracts[1] of papers read at the Seventh Annual Meeting together with the European Club for Paediatric Research Vienna Austria August 26–30 1968*

Donald Fraser (Department of Paediatrics University of Toronto and the Research Institut the Hospital for Sick Children Toronto Canada intr by W Swoboda) *Physiology of parathyroid hormone and certain pathophysiological applications in paediatrics*

In recent years advances in protein chemistry have made possible the purification and characterization of parathyroid hormone and have stimulated study of its biochemistry physiology and pathophysiology The most important physiological role of PTH is to maintain the level of ECF calcium This is accomplished mainly by mobilization of bone calcium but also by increasing intestinal absorption and renal tubular reabsorption of calcium Parathyroid hormone also causes phosphaturia by a direct action on the renal tubules The calcium mobilizing action of PTH requires the presence of vitamin D but the phosphaturic action is apparently independent of vitamin D

The mode of action of purified PTH at the cellular and subcellular levels is presently an active field of biochemical study Recent evidence of Chase & Aurbach (1) and associates and of Russel *et al* (2) suggests that the phosphaturic action of PTH is mediated by 3 -5 AMP (cyclic AMP)

In the normal situation the level of circulating PTH is controlled by the level of ionized calcium in the blood by a feed back mechanism hypocalcaemia stimulating PTH secretion hypercalcaemia reducing PTH secretion Magnesium ion apparently has a similar but less marked action the physiological significance of which is still in question

Only radioimmunoassay is sufficiently sensitive to allow direct measurement of the levels of PTH in the blood and only now are suitable adaptations starting to become available to measure human PTH Nevertheless the physiological principles enumerated above have allowed much to be learned about the pathophysiology of the parathyroids in many clinical conditions

*Pathophysiological applications in paediatrics*

For many years the group in Toronto has been interested in the role of the parathyroids in various types of rickets Interest in this aspect commenced when it was found that experimentally induced hypercalcaemia caused a transient correction of the excessive phosphaturia in vitamin D refractory rickets but not in cystinosis (3) Suppression of parathyroid hyperactivity was one interpretation Hypercalcaemia also corrected hyperaminoaciduria in vitamin D dependent rickets (pseudodeficiency rickets) This led us with our colleague Dr Charles Scriver of Montreal to a study of vitamin D deficiency rickets (4) Our joint observations suggested that there were three stages of vitamin D deficiency in infancy depending upon the extent and duration of deprivation and that the aminoaciduria and phosphaturia

[1] These papers were read at sessions on the 28th and 9th of August.

11 Mortensen E Studies on the osmotic fragility of normal human erythrocytes I *Acta Med Scand* *173* 683 1963
12 Nordøy A Bremer J Myhre E & Solheim S Studies on glycolysis in hereditary non sphero cytic hemolytic disease *Acta Med Scand* *172* 67 1962

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D. Gekle (Univ. Kinderklinik, Würzburg, Germany, intr. by W. Swoboda): *Micropuncture investigations of the effect of thyrocalcitonin and parathormone on the kidney*

In spite of a great number of investigations on the mechanism of action of parathormone (PTH), its biochemical action and its site of impact within the cell are not yet fully understood. Particularly the renal actions of the hormone appear confusing. Its phosphaturic effect has been known for a long time. But there are furthermore reports on an enhanced urinary excretion of sodium, potassium and water under the influence of PTH. Since such an influence of PTH would be very important, this phenomenon was investigated in our experiments using micropuncture methods. We have studied the sodium and fluid reabsorption from the proximal tubule of the rat. The results demonstrate an inhibitory effect of PTH on proximal tubular sodium and fluid reabsorption. This effect results in an increase in urine flow and enhanced excretion of sodium and potassium. In further experiments we explored the influence of PTH on the tubular reabsorption of amino acids. The data showed under PTH an inhibitory effect on the transtubular reabsorption of amino acids. The excretion of amino acids increased significantly, except the dicarboxylic amino acids aspartic and glutamic, which have a common transport system in mammalian kidney. Glomerular filtration rate did not change. It is suggested that the hormone exert an effect on tubular cell membranes.

In other series of investigations we studied the influence of exogenous and endogenous thyrocalcitonin (TCT) on the phosphate reabsorption in the rat kidney. From each puncture the concentration ratio of tubular fluid (TF)/plasma (P) for phosphate was determined. The experiments were carried out with normal and with parathyreoidectomized animals. The normal value of TF/P phosphate ratio in the proximal tubule is 0.71 ± 0.09. Parathyreoidectomized rats have a TF/P phosphate from 0.32 ± 0.13. After s.c. injection of exogenous TCT the TF/P phosphate in parathyreoidectomized animals was 0.64 ± 0.14. The effect of endogenous produced TCT (by s.c. injection from TSH) was 0.63 ± 0.02. From these results we can conclude that TCT like PTH inhibits the reabsorption of phosphate in the proximal renal tubule.

L. Corbeel, P. Casaer, P. Malvaux, J. Lormans & N. Bourgeois (Service de Pédiatrie, Université Louvain, Belgium): *Congenital hyperparathyroidism*

A one month-old female baby was admitted to the hospital because of dehydration and hypotonicity. It was the first child of healthy but consanguineous parents. Analysis of the serum revealed hypercalcaemia.

In the table we give the range of values for Ca and P in blood and urine during the first week of observation.

| *Blood* | | *Urine* | |
|---|---|---|---|
| Ca mg | 16–20 | cc/24 h | 250–300 |
| P mg | 2.5–3 | Ca mg/kg/24 h | 8–11 |
| Pase U.B. | 16 | P mg/kg/24 h | 65–85 |
| | | Tubular resorption | 45 |

The radiological examination of the bones revealed an extensive demineralisation with multiple fractures. Urinanalysis showed aminoaciduria with pronounced hydroxyprolinuria. Parenteral fluid therapy during a period of 8 days induced a striking improvement of the clinical condition with a decrease of serum calcium (see Fig. 1), an increase of the serum phosphorus and a weight gain of 1 kg within 28 days. On the 29th day after admission the patient developed bronchopneumonia. The serum Ca increased to 30.5 mg and therefore a partial parathyroidectomy was performed. The serum calcium now fell to 9 mg within 5 days, producing symptoms of tetany. Ca, vit. D and finally parathormone were therefore administrated. 13 days after the partial para-

characteristic of stages II and III were due to secondary hyperparathyroidism

There are indications from the literature that calcium homeostasis *is not normal in osteopetrosis* and we have *recently confirmed* this finding in the severe recessively inherited form of that *condition* In the patient, there was virtually complete refractoriness *to the cal*cium mobilizing actions *of PTH* and vitamin D at the bone *level* whereas responses to these substances were observed at the renal and intestinal levels (5)

*Without* question the rapidly advancing knowledge *of PTH physiology and of im*munoassay techniques will *find many* additional applications in clinical disturbances of calcium and bone metabolism

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C A Anast (Department of Pediatrics University of Missouri Columbia Missouri USA intr by W Swoboda) *Thyrocalcitonin—a newly recognized hypocalcemic factor*

Thyrocalcitonin is a polypeptide derived from the thyroid C-cells in mammals that lowers the concentrations of calcium and phosphorus in the plasma *Embryologically* the thyroid C cells are derived from the ultimo branchial body In contrast to mammals the ultimobranchial body of lower vertebrates (fishes birds, amphibians) remains as a distinct structure separate from the thyroid gland The ultimobranchial body of these lower forms contains a substance that is hypocalcemic in mammals and hypothetically this factor is the hormonal homologue of thyrocalcitonin

The molecular weight of porcine thyrocalcitonin is approximately 4500 The administration *of less than 1 0* microgram of the purified polypeptide induces a rapid fall in the plasma calcium and phosphorus levels in rats *In vivo* and *in vitro* studies both show that thyrocalcitonin exerts its *hypocalcemic effect by sup*pressing bone resorption The long term administration of thyrocalcitonin to rats results in increased trabecular bone in the metaphyses The fact that thyrocalcitonin is active in parathyroidectomized rats indicates that it does not produce its effect by *inhibiting parathyroid gland* secretion or by inactivating parathyroid hormone The secretion of thyrocalcitonin appears to be controlled by a negative feed back mechanism whereby hypercalcemia stimulates the secretion of this hypocalcemic factor

Thyrocalcitonin has been shown to be of physiologic significance in calcium homeostasis in experimental animals Studies in the rat indicate that the thyroid gland functions as a potent protective mechanism against the toxic effects produced by parathyroid hormone and that exogenous thyrocalcitonin can replace the thyroid gland in this regard In addition thyrocalcitonin has been demonstrated to counteract hypercalcemia induced by exogenous calcium loads

*Hypocalcemic* activity has been found in crude extracts of human thyroid glands and interestingly increased activity has been found in the thyroid glands of patients with pseudohypoparathyroidism as well as in patients with medullary carcinoma of the thyroid Available evidence indicates that the thyroid gland in humans plays a significant role in counteracting artificially induced hypercalcemia, presumably by secreting thyrocalcitonin Further work is needed to evaluate *the physiologic* significance of this factor in man It is possible that new concepts regarding calcium phosphorus and bone metabolism may emerge as more knowledge of the biologic nature of thyrocalcitonin is acquired

escaped notice Although it has been known for some time that high doses of fluoride cause osteomalacic changes in the bone of various species the conspicuous presence of ectopic bone in chronic fluorosis (tendons and ligaments) has not been linked with this osteomalacic process Since calcification of ligaments and tendons is also a feature of vitamin D resistant rickets—especially in untreated adults—it may be that these ectopic calcifications in fluorosis are consequent upon a chronic osteomalacic condition rather than a direct result of high levels of fluoride

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R D G Milner (Department of Biochemistry University of Cambridge England) *Insulin secretion from foetal and postnatal rabbit pancreas studied* in vitro

Insulin is present in rabbit pancreas from the 18th day of foetal life onwards (3 4) The concentration of immunoreactive insulin in foetal pancreas from day 22 is of the same order as that found in 6–12 week postnatal animals Insulin is pres nt in foetal plasma from day 20 when the concentration is consistently lower than that in maternal plasma From day 20 to day 30 there is a fall in maternal plasma insulin concentration but no significant change in the foetal concentration so that on day 30 the foetal plasma insulin conc ntration is significantly higher than the maternal concentration

Pi ces of pancreas from 24 and 30-day rabbit foetus s and 1-day and 6–12 week animals were incubated *in vitro* and insulin release was studied by a modification of the method of Coore & Randle (2) Measurements of basal insulin release were made in medium containing 0 6 mg/ml glucose Insulin release was increased at each age studied by 3 mg/ml glucose 5 mM L leucine 5 μg/ml glucagon (in the presence of 3 mg/ml glucose) 60 mM potassium and 10 M ouabain

In the rabbit granulation of the β cell visible by light microscopy occurs first at the time of delivery or in the first week of postnatal life (1 3) The presence of insulin in foetal tissues from day 18 in foetal plasma from day 20 and the release of the hormone from foetal β cells from day 24 all suggest that insulin of fo t.l origin plays a part in foetal metabolism and that morphological maturity is not a prerequisite for functional competence in the foetal β cell

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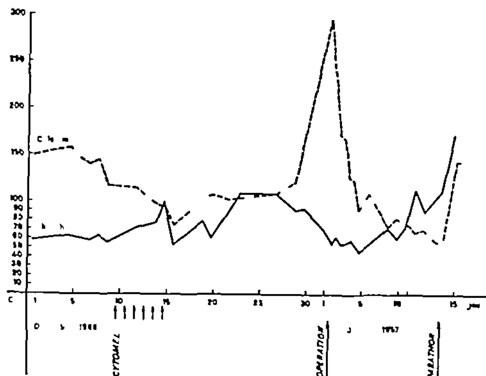
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G Chiumello (Department of Pediatrics University of Milan Italy intr by F Sereni) *Relationship between obesity chemical diabetes mellitus and insulin secretion in children*

The obese adult is characterized by an excessive insulin release following pancreatic beta cell stimulation and by an increased incidence of diabetes mellitus as defined by abnormality of glucose tolerance Obesity in the child on the other hand is uncommonly associated with abnormalities of glucose tolerance

The current investigation was designed to compare the insulin response to glucose administration p r os and i v in 4 groups of children of similar age (7 to 12 years) Group I children of normal weight without family history of diabetes mellitus Group II children of normal weight with strong family history of diabetes Group III obese children without



thyroidectomy the baby died from bronchopneumonia

Necropsy showed extensive bronchopneumonia normal kidneys Histological examination of three parathyroids revealed a hyperplasia of the water clear cells in the three glands The thyroid was treated with Mallory blue to demonstrate metachromasia of the parafollicular cells there was no difference between the histological aspect of the patient s thyroid and that of a control baby of the same age Radiological examination of the bones showed a marked remineralisation and disappearance of the multiple fractures Hypoparathyroidism of the mother could be eliminated as a cause of the hyperparathyroidism of the infant

A congenital defect of the secretion of parat hormone or of a mechanism which controls this secretion is postulated

R Steendijk (Departments of Paediatrics and Histology University of Amsterdam Holland) *Microscopic aspects of vitamin D resistant rickets and skeletal fluorosis*

In previously reported studies (1) of compact bone from patients with vitamin D resistant rickets (familial or essential hypophosphatemia) microradiographic examination of undecalcified bone sections revealed a lack of mineral around osteocyte lacunae and their canaliculi On histological examination of decalcified sections a lesion of the bone matrix was observed at these sites This lesion was characterized by an abnormally strong affinity for azure II an interruption of the normal lamellar pattern and a globular appearance of the matrix In bones from several patients with other types of rickets and osteomalacia these abnormalities were either absent or occurred only sporadically

Recently it has been found (2) that the same pattern of perilacunar low mineral density is present around many lacunae in bone from patients with chronic skeletal fluorosis Examination of fluorotic compact bone by the methods used in the previous study revealed that the matrix lesion—as found in decalcified sections—was also present

The occurrence of an identical lesion in two diseases of different etiology raises the question of possible similarities in the skeletal pathology of vitamin D resistant rickets and chronic fluorosis which hitherto might have

(*b*) Alpha receptor blocking agents which compete with norepinephrine for the alpha receptor sites reverse the inhibition of insulin the insulin secretion increases or is even higher than expected for the given glucose level
(c) Also in normal healthy adults insulin secretion in response to glucose load increases under the influence of alpha receptor blocking agents

It can be concluded that norepinephrine plays a role in the regulation of insulin secretion in patients with pheochromocytoma as as well as under normal conditions The time coincidence of the drop of urinary norepinephrine excretion and the rise of glucose and insulin are in agreement with this conclusion

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B Weber H J Quabbe H Helge & E Werner (Endocrinology Study Group Pediatric Service Free University Berlin Germany)


*Growth hormone release after glucagon administration in normal and obese children*

Growth hormone (GH) plasma levels were investigated following glucagon injection (1 mg i v) in normal obese and—for comparison—in diabetic children Contrary to the results in adults (1) the majority of normal children (11 out of 15) do react to glucagon application with a prompt elevation of GH-concentrations (mean values of the responders 1 7 to 6 5 ng/ml of the whole group 3 4 to 6 7 ng/ml) within 10 min an increase of 2 ng/ml or more being considered as a positive response

Among 12 obese children exhibiting a relative overweight of more than 20 % only 6 among 9 diabetics only 2 could be regarded as responders GH increase was less pronounced in the obese (1 8 to 4 4 ng/ml at 10 min $n=6$ responders 2 2 to 3 5 ng/ml (mean values of $n=12$)) and quantitatively normal in the two diabetics GH maxima were reached at 30 min in normal (8 2 ng/ml ($n=11$) 7 1 ng/ml ($n=15$) and diabetic children at 10 min in the obese

In spite of the glucagon induced elevation of blood glucose values GH *release* can be demonstrated in children of any age Its effect in newborns has recently been shown by others (2) However the mode of action of glucagon with respect to GH secretion so far is unknown The described effect appears to be provoked by a nonspecific stresslike stimulation, possibly transmitted by hypothalamic adrenergic mechanisms as suggested by some experiments in rats (3) and in man (4) Epinephrine concentrations in peripheral blood however do not seem to influence glucagon induced GH release since a growth hormone increase likewise occurred in one of our children after total adrenalectomy The high frequency of positive GH responses in children who in this respect differ from adults corresponds to the much higher incidence of GH elevations after *another stressful* procedure e g venipuncture which in children quite often, in adults only *rarely induces* a *GH* increase in plasma

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family history of diabetes Group IV obese *children with strong family history of diabetes* There is no difference in the degree of obesity in patients of groups III and IV

The data showed

1 The incidence of chemical diabetes is higher in the group with strong family history of diabetes,
2 In obese children, like in adults hyperinsulinism is present when glucose tolerance is normal this is associated with pancreatic hyperresponsiveness to glucose
3 The development of glucose intolerance is associated with impairment of insulin production probably related to a genetically conditioned incapability of betacells to respond to various stimuli (high incidence in the obese with family history of diabetes)
4 Diabetes develops in the face of relative insulin deficiency regardless of the absolute *plasma insulin concentration*
5 With the limitation of the number of subjects examined obesity per se cannot be interpreted to represent a state of latent diabetes but could be an additional stress in subjects who carry an inherited tendency to the disease
6 Obesity does not alter the basic defect in diabetes mellitus in childhood which is a deficiency of betacells function

Ruth Illig & W Ziegler (Departments of Pediatrics and Internal Medicine University of Zurich Switzerland) *Blood glucose immunoreactive insulin norepinephrine and alpha receptor blocking agents*

In 1966 Porte *et al* (1 2) showed that epinephrine and norepinephrine infusions in man inhibit insulin release from the pancreas These observations led us to determine blood glucose (glucose *oxidase method) and im*munoreactive insulin (IRI) in plasma (3) after an oral glucose load in 3 patients with norepinephrine producing tumors (2 tumors localized in the adrenal one metastatic tumor) and in 3 healthy volunteers of normal body weight and without a family history of diabetes Oral glucose tolerance tests (50 g glucose per m body surface area) were performed before therapy under oral treatment with phenoxybenzamine, and alphareceptor blocking agent and in 2 patients after surgical removal of the adrenal tumor The effect of alpha receptor blockade was judged by a fall of high blood pressure and/or by an increase of urinary excretion of norepinephrine (4)

Blood glucose rose very high and remained over 120 mg per 100 ml after 2 hours in all 3 pheochromocytoma patients under treatment with phenoxybenzamine, there was no change or only a slight improvement after tumor removal glucose curves became normal

Before therapy IRI in response to glucose ingestion did not increase in a 13 year-old girl with extreme high plasma concentrations of norepinephrine (maximal value during vena cava catheterisation 216 $\mu$g/l plasma) and the rise of IRI was inadequate with respect to their blood glucose values in the 2 other patients Alpha receptor blockade enhanced the IRI response to oral glucose load in the 3 cases with pheochromocytoma and also in 2 of the 3 healthy controls in 2 patients IRI reached even excessively high values After operation of the tumors plasma IRI was adequate for the glucose increase

Fractionated collection of urine before and after oral glucose load (9 times in 3 controls and in 1 patient) revealed a drop of urinary norepinephrine (expressed in $\mu$g/g creatinine) during the first 45 minutes after glucose intake in all 9 instances in the untreated state as well as under alpha receptor blockade

Our results can be summarized as follows

(*a*) In 3 patients with pheochromocytoma and high plasma levels of norepinephrine the *release of insulin is insufficient in* response to elevated blood glucose The degree of insulin inhibition seems to depend on the concentration of plasma norepinephrine

(b) Alpha receptor blocking agents which compete with norepinephrine for the alpha receptor sites reverse the inhibition of insulin the insulin secretion increases or is even higher than expected for the given glucose level

c) Also in normal healthy adults insulin secretion in response to glucose load increases under the influence of alpha receptor blocking agents

It can be concluded that norepinephrine plays a role in the regulation of insulin secretion in patients with pheochromocytoma as as well as under normal conditions The time coincidence of the drop of urinary norepinephrine excretion and the rise of glucose and insulin are in agreement with this conclusion

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8 Weber H J Quabbe H Helge & E Werner (Endocrinology Study Group Pediatric Service Free University Berlin Germany) *Growth hormone release after glucagon administration in normal and obese children*

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H Schedewie F Haour & J Bertrand (Unite de Recherches endocrinniennes et metaboliques chez l enfant (INSERM) Lyon, France) *Growth hormone (GH) levels in hypothyroid children and preliminary results with a new growth hormone provocation test*

GH levels were determined in 10 hypothyroid infants and children prior to treatment In six of them GH was re estimated after treatment when clinical signs had receded In all those where GH levels were subnormal before treat ment they had risen significantly after treat ment

Release of GH was stimulated by infusion of arginine or by insulin induced hypoglycemia *Only one provocative agent was given in the* majority of cases In several children stimula tion was achieved by a new method using arginine and insulin simultaneously

Since arginine is a potent stimulator of GH release, despite its not infrequent hypergly cemic effect it would seem that the stimulus it provides to GH release is of an entirely dif ferent nature compared with that which follows hypoglycemia It therefore seemed logical to attempt to provoke GH release with a combi nation of the two stimuli One aim was to seek a more powerful stimulus than those already in use, none of which is completely satisfac tory

In the second place it was hoped that com parison of the metabolic effects obtained using the three different stimuli might throw some light on their mode of action With this in mind a concomitant study of plasma insulin cortisol and NEFA levels in the hypothyroid and in other pediatric as well as in adult sub jects is currently under way

$^{131}$I labelled GH has been prepared by the method of W M Hunter & F C Greenwood *J Biochem* 89 114 1963 Plasma GH has been estimated by the method of D S Schalch & M L Parker *Nature 203* 1141 1964 The sensitivity obtained being such that about 1 mg/ml of HGH can be measured

M Zachmann (Department of Paediatrics University of Zurich, Switzerland mtr by G Murset) *Influence of human growth hormone on plasma and urine amino acids*

It is known that the concentrations of some amino acids in plasma are significantly lower in untreated hypopituitary dwarfs than in con trol children (1) After short term treatment of hypopituitary dwarfs with human growth hor mone (HGH) the concentration of serum-a amino nitrogen raises (2) To study whether this increase is caused by a generalized or a selective elevation of plasma amino acids we have analyzed amino acid concentrations in plasma and urine of 6 hypopituitary dwarfs before and after 3-5 days of HGH administra tion (2 mg/m /day) A rigidly constant diet was maintained throughout the test period of 6-10 days duration

The values before treatment were signifi cantly below the normal range for this age group for threonine, serine proline glycine and alanine and were raised into the normal range after HGH administration The concen trations of taurine the sum of aspartic glu tamic acid and asparagine glutamine methio nine, isoleucine, tyrosine phenylalanine orni thine and lysine were within the normal range before but increased also after treat ment On the other hand the values of citrul line leucine histidine and arginine were within the normal range and were not influ enced by HGH The mean per cent increase after treatment was largest for threonine fol lowed by serine glycine and methionine

Amino acid concentrations in urine and the amino acid clearances did not show consistant changes Although this point requires further investigation it seems therefore unlikely that renal mechanisms are responsible for the changes in plasma amino acid concentrations

As shown previously some plasma amino acids increase significantly in correlation with the increasing testosterone excretion during puberty in the male (1 3) These include glu tamic acid valine, methionine isoleucine leu

cine and phenylalanine None of these amino acids was among those significantly lowered in the patients of the present study and with the exception of methionine none of these increased considerably after treatment with HGH It seems therefore that HGH has different effects on the metabolism of amino acids than testosterone

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2 Prader A Zachmann M Poley J R & Illig R *The metabolic effect of a small uniform dose of human growth hormone in hypopituitary dwarfs and in control children* I Nitrogen α amino N creatine creatinine and calcium excretion and serum urea N α amino-N inorganic phosphorus and alkaline phosphatase *Acta Endocr* (Kbh) 57 115 1968
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tatti R M Blizzard & C J Migeon (Instu of Child Health London England and Johns Hopkins Hospital Baltimore Md A intr by A Prader) *Production rate of icle stimulating hormone (FSH) in adult man males using a double isotope technique*

: radioimmunoassay technique and materi of Midgley (1) were used To measure im nological FSH in urine a technique of al rol precipitation of FSH from 5 ml of urine s used (2) and recoveries of 2nd IRP HMG re 95–100 between the levels of 6 6–40 0 U/ml

Immunologically pure [1] I FSH (LER 780) doses of 0 75–2 0 uC (specific activity = 87 :/ μg) were injected intravenously into 6 adult iles (one subject was studied twice) These tients were taking prophylactic potassium lide during the period of the study

Results were as follows

1 *Recoveries of injected radio-activity* These ranged from 82 0–94 6 in 3 days in 6 studies and 57 9 in the 7th study
2 *Identification of labelled protein in excreted radioactivity*
   (*a*) Alcohol precipitation = 29–41 5 of total radioactivity
   (*b*) Gel filtration = 27 1–34
   (*c*) Antibody binding properties = 73 3–89 2 of protein bound [131]I
3 *Stable FSH excreted in urine* This varied from 7 6–11 1 IU/day in 6 of the studies and was 4 5 IU/day in the 7th case The day to-day variability did not exceed 2 5 IU/day
4 *The production rate* This can be calculated from the following formula

$$\frac{\text{Dose of immunologically reactive FSH injected}}{\text{Specified activity of urinary product} \times \textit{time in days}}$$

The production rates were 18 8 18 8 24 3 2o 7 26 8 and 34 6 IU/day

Of importance is the observation that about one third of pituitary FSH injected was excreted in an immunologically unaltered state

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1 Mid ley A R J Clin Endocr 27 295 1967
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F Peter L Szentsayi Nagy G Szombathy & J Bánfi (Dept of Pediatrics Dept of Surgery Univ of Debrecen Medical School and Labor of Peterfy Hospital Budapest Hungary intr by W Swoboda) *Immunopathological studies of thyroid disorders in childhood*

In recent years chiefly on the basis of case reports the importance of the immunological processes has repeatedly been emphasized in the pathogenesis of thyroid disorders in childhood Five hundred antithyroid antibody tests were carried out between 1961 and 1968 We

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Rath R M Blizzard & C J Migeon (Institute of Child Health London England and The Johns Hopkins Hospital Baltimore Md USA intr by A Prader) *Production rate of follicle stimulating hormone (FSH) in adult human males using a double isotope technique*

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F Peter L Szecsenyi Nagy G Szombathy & J Banfi (D pt of Pediatrics Dept of Surgery Univ of Debrecen Medical School and Labor of Peterfy Hospital Budapest Hungary intr by W Swoboda) *Immunopathological studies of thyroid disorders in childhood*

In recent years chiefly on the basis of case reports the importance of the immunological processes has repeatedly been emphasized in the pathogenesis of thyroid disorders in childhood Five hundred antithyroid antibody tests were carried out between 1961 and 1968 We

are reporting on the investigations of the following groups Diabetes mellitus (25 children 33 investigations), hyperthyroidism (5 13), hypothyroidism (15 19) endocrinopathy (20 23) collagenosis (8 10), euthyroid goitre (228 290)

The antibody investigations were performed according to the modified Oakley & Fulthrop version of the original Goudi *et al* method Material for the histological investigation was obtained by strumectomy (13 cases) the cytological analysis was carried out by means of the Soderstrom technique (32 cases) The antibody determination was strongly positive in 65 cases of patients with goitre The histological analysis established the presence of lymphocytic thyroiditis only in 2 cases A significant decrease of the antibody titer was observed in 17 children when repeated estimations were done We obtained incongruent results by a histological analysis compared with cytological ones

B Salle (Hopital Edouard Herriot Lyon, France intr by R François) *Histologic study of gonads in male pseudo hermaphroditism*

Twenty eight biopsies of gonads obtained from 17 children aged between 3 months and 19 years and who presented male pseudo hermaphroditism were selected for this study

The cases are divided into three groups

Testicular Feminizing syndrome 6
Male pseudo hermaphroditism 8
Mixed Gonadal Dysgenesis 3

The most interesting results are obtained from the gonads of mixed gonadal dysgenesis The serial sections show proof of a tumour composed of germinatives cells in the streak in two cases and in the opposite testis in one case These tumours have no calcifications nor Call Exner Bodies, they are different from Gonadoblastoma described by Scully and are histologically similar to seminoma This sort of tumour has never been found before puberty Apart from the theoretical interest, such a finding poses the problem of removal of the streak as well as of the opposite testis in mixed gonadal dysgenesis to avoid the future development of generalized seminoma from the nest of germ cells

J M Saez, F Frederich & J Bertrand (Unite de Recherches endocriniennes et metaboliques chez l infant (INSERM) Lyon France intr by M Cathro) *Testicular function in normal children and in children with male pseudohermaphrodism*

Plasma levels of testosterone (T) and of dehydroepiandrosterone sulphate (DHAS) have been measured in normal children and in children with male pseudo hermaphrodism both before and after stimulation with human chorionic gonadotrophin (HCG) Testosterone has been measured by a technique involving labelling with tritiated acetic anhydride and DHAS by means of gas liquid chromatography

The mean concentration of plasma T in normal children aged 2 to 13 $^1/_2$ years, all of them without any clinical evidence of puberty was 31 8 ± 10 (S D) mμg/100 ml In the group of subjects studied, no significant difference was found between the younger and the older children After testicular stimulation with HCG the mean plasma testosterone value was 554 ± 121 (S D) mμg/100 ml

The concentration of DHAS was very low less than 15 μg/100 ml in the 10 youngest subjects all of whom were less than 8 years old Thereafter the concentrations were found to rise progressively with age and after the age of 11 years but before puberty the mean value was 67 2 μg/100 ml The mean concentration of DHAS in the 10 youngest children was 5 4 ± 2 5 (S D) μg/100 ml before HCG was given After HCG administration the mean level rose to 13 9 ± 5 5 (S D) μg/100 ml The difference is statistically significant ($p$ 0 02) These results

Table 1 *Upper arm composition during puberty*

| | Girls Breast development rating | | | | Boys Volume of each testis ml | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3+4 | 5 | 2–3 | 4–5 | 6–7 | 8–12 | 13–> |
| No of children | 6 | 10 | 30 | 9 | 18 | 13 | 11 | 13 | 12 |
| Age yrs Average | 12.0 | 12.4 | 12.9 | 13.2 | 12.4 | 12.7 | 13.5 | 13.8 | 14.8 |
| Range | 11.2–13.1 | 11.6–13.8 | 11.5–14.3 | 12.6–14.0 | 11.3–13.8 | 11.4–14.9 | 12.2 15.0 | 12.4–16.2 | 13.4–16.6 |
| Circumference mm | 213 | 209 | 219 | 255 | 200 | 226 | 218 | 224 | 244 |
| rps skinfold m | 6.8 | 5.9 | 6.2 | 8.9 | 4.1 | 7.3 | 6.0 | 5.0 | 5.1 |
| ceps skinfold m | 11.3 | 9.5 | 9.3 | 13.6 | 7.1 | 11.6 | 9.4 | 7.6 | 8.0 |
| tal surface rea cm² | 37.2 | 34.8 | 35.4 | 51.9 | 32.2 | 39.5 | 38.3 | 40.4 | 47.6 |
| ne + muscle rea cm | 27.5 | 27.1 | 30.3 | 38.4 | 26.5 | 30.9 | 30.3 | 33.5 | 39.8 |
| t + skin area, m | 9.7 | 7.7 | 8.1 | 13.5 | 5.6 | 8.6 | 8.1 | 6.9 | 7.7 |

ggest that there is testicular secretion either DHAS itself or of a precursor which is onverted into DHAS by peripheral tissues

Seventeen children with male pseudo-hermaphrodism aged 1½ to 10 years have been tudied The mean concentration of T under asal conditions was 39.4±8.4 (S.D.) mμg/100 ml which is not different from that found in normal children In the same subjects the mean value for testosterone following HCG at 167±157 mμg/100 ml was significantly lower than that found in normal children under the same conditions On the other hand if the results from the individual patients are compared with the results from normal children following HCG seven patients showed an abnormally low rise in testosterone in the other 10 subjects the response was normal The mean DHAS levels before and after HCG were 6.7±3.2 (S.D.) and 14.8±5.7 μg/100 ml respectively Both means are higher than those found in the controls but the differences are not statistically significant However in two patients in whom the response of T to HCG was very poor DHAS did not increase at all

In eleven patients the sensitivity of the end organ structures to androgens was studied by measuring the nitrogen balance before and during the administration of testosterone propionate All patients responded by increasing nitrogen retention

J J van der Werff ten Bosch (Department of Endocrinology Growth and Reproduction Medical School Rotterdam Holland) *Puberty and body composition*

A cross sectional study was made of children just before or in puberty Measurements included skinfolds over biceps and triceps subscapular skinfolds and the circumference of upper arm (mid way at level of skinfold measurements) Pubertal signs were noted In boys a change of testis size from 3 ml or less to 4 or 5 ml was the most frequent earliest sign of puberty in girls onset of breast development nearly always preceded pubic hair These earliest signs were used for grouping the observations

This report is concerned with fat (plus skin) and muscle (plus bone) at different stages of puberty Approximations for these components in a cross sectional area were calculated by assuming that the arm circumference is a

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Z Laron & A Pertzelan (Pediatric Metabolic and Endocrine Service Beilinson Hospital Univ Med School Tel Aviv Israel) *Clinical use of the antiandrogen cyproteron (SH-80 881) in the pediatric age group*

Cyproteron was administered in doses of 100–200 mg/day to 17 patients (14 females and 3 males) ranging in age from 6 ⁶/₁₂ to 18 ⁶/₁₂ years According to the diagnosis and indications for treatment they belonged to the following groups

I Precocious sexual development (5 pts)
II Cong adrenal virilism (6 pts)
III Cong adrenal virilism post puberal (2 pts)
IV Increased growth or hirsutism

The period of treatment ranged from 4 to 13 months

The results can be summarized as follows The amount and degree of *acne* was reduced in all patients to whom they were disturbing *Hirsutism* was difficult to evaluate *Sexual drive* as evidenced by masturbation was stopped *Menstruation* was not affected nor was body weight *Linear growth* was not significantly influenced however there was a tendency for decrease in growth velocity *Skeletal maturation* was unchanged but considering that most patients had an advanced bone age it is possible that maturation was slowed

There was no change in the daily excretion of the urinary 17KS but there was a marked increase in the total urinary 17 OHCS without clinical signs of hypercorticism An exception were two patients who developed striae after one year of continuous treatment They had no moon face or elevated blood pressure It is assumed that most of the total 17 OHCS are metabolites of the drug administered

W Blanck (Universitats Kinderklinik Hamburg Germany intr by J Bierich) *Estimation of 17-oxo-steroids by thin layer-chromatography*

We are very sceptical about the diagnostic value of a 17-oxo-steroid estimation resulting from a group-reaction in the crude urinary extract Especially in infancy the total quantity of 17-oxo steroid metabolites as found after chromatographic separation amounts between 5–80 of the value found by one of the usual group estimations

By using a quick and uncomplicated purification and first separation of the urinary extract on one silica gel plate many unspecific chromogens can be eliminated After glucuronidase sulfatase hydrolysis and solvolysis or acid hydrolysis the crude extract is chromatographed on the same plate in 4 consecutive solvents of ascending polarity (benzene benzene-chloroform 1 1 chloroform chloroform acetone 9 1) The $C_{21}$ $C_{19}O_2$ and the $C_{19}O$ steroids are roughly separated After elution of the $C_{19}O$ and the $C_{19}O_2$ fraction the important ratio of 11 deoxy /11-oxy 17-oxo-steroids ($=C_{19}O$ / $C_{19}O_2$-ratio) can be stated This step of purification and elution takes 4 hours for 6 samples but the fractions are still contaminated by some unspecific chromogens

For further fractionation a separation procedure of the main three $C_{19}O_2$ steroids an alumina/kieselguhr (1 1) by benzene/methylene chloride (4 times) is presented the specific behaviour of DHA in this system is useful for the identification and true estimation of this metabolite Four $C_{19}O_2$-steroids are separated on alumina/silica gel (1 1) in the solvent system 3 iso-propanol in methylene chloride (3 times) Urorosein may be separated from the $C_{19}O$-steroids on freshly activated—still warm—silica gel by one run in ether

The chromatograms are demonstrated normal values problems of hydrolysis and criteria of reliability are discussed

D Knorr & O Butenandt (Universitats Kinderklinik Munich Germany) *Gas liquid chromatographic investigations in homozygote and heterozygote persons with congenital adrenal hyperplasia*

17 alpha hydroxyprogesterone lies immediately before the enzymatic defect in the steroid path

circle, and that the sum of the two arm skinfolds represents four times the thickness of an even layer of skin and its subcutaneous fat Some of the data appear in the Table

In boys the increase in testis size to 4 or 5 ml was associated with a rise in skinfold thickness particularly over the biceps, and an increase in the amount of bone plus muscle At larger testis sizes the classical loss of fat occurred in the arm skinfolds whilst the subscapular skinfold underwent a gradual rise from the very beginning of puberty onward

In girls a loss of arm fat occurred initially which was followed by a steep rise late in puberty subscapular fat likewise rose during the latter stages of puberty The bone and muscle component remained unaltered at first and increased between breast development stages 2 and 3

It is concluded that the very first effects of sex hormones in the boy consist of accumulation of subcutaneous limb fat along with some muscle growth fat loss and more pronounced muscular development do not occur until the testes have reached a volume of over 5 ml It is curious to note that the boy with testes of 4 or 5 ml (i e clearly in puberty ) resembles the girl which has not yet started to come in puberty

These initial changes in boys have not before been reported in longitudinal studies One reason why they may have passed unnoticed is the fact that the pubescent boy passes very quickly through the various stages of testicular development Testes may grow from 3 ml to 10 ml within a period of 12 months with an interval of three months between observations usually employed in longitudinal studies the short phase during which the above changes occur may be missed

An initial rise in skinfold thickness has been repeatedly observed by the author in girls during treatment with androgens

J M Tanner & D Gupta (Institute of Chi Health London England) *A longitudinal stu of the excretion of individual steroids duri adolescence*

The urinary excretion of twenty one indiv ually-estimated $C_{19}$ and $C_{21}$ steroids has bee studied at 6 monthly intervals in 18 boys fro 9 to 15 and 10 girls from 9 to 14 Growt skeletal maturity and the development of s ondary sex characteristics were followed s multaneously Separation and estimation of t steroids was done by sephadex extraction e zyme hydrolysis and solvolysis, one stage pap chromatography thin layer chromatography a gasliquid chromatography

There were marked and consistent diffe ences between children in the excretion most steroids before adolescence but so children who were high excretors before a olescence of one steroid moved to be relative low excretors in adolescence At adolescen there were rapid increases in the excretion pa ticularly of testosterone, androsterone, d hydroepiandrosterone and 11 $\beta$ hydroxyandr sterone in boys the chief rise was betwe stages 3 and 5 of the genitalia developme (Androsterone from mean of 36 $\mu$g/kg/24 hr stage 4 to 73 $\mu$g/kg/24 hr in stage 5 testo terone 0 37 $\mu$g/kg/24 hr in stage 3 0 49 kg/24 hr in stage 4 and 0 57 in stage 5 ) T excretion of aetiocholanolone rose consid ably less There was no rise in testosterone e cretion in girls (0 16 $\mu$g/kg) and no rise in e testosterone excretion in either sex Sex d ferences in excretion of these steroids becar marked after age 12 Both age trends and s differences were more pronounced when excr tion was related to skeletal maturity than wh related to chronological age No marked s differences occurred in the excretion of corti metabolites during adolescence The glucur nide to sulphate ratio of the 11 deoxy 17 ox steroids decreased consistently with increasi age

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D Knorr & O Butenandt (Universitats-Kinderklinik Munich Germany) *Gas liquid chromatographic investigations in homozygote and heterozygote persons with congenital adrenal hyperplasia*

17 alpha hydroxyprogesterone lies immediately before the enzymatic defect in the steroid path

circle and that the sum of the two arm skinfolds represents four times the thickness of an even layer of skin and its subcutaneous fat Some of the data appear in the Table

In boys the increase in testis size to 4 or 5 ml was associated with a rise in skinfold thickness particularly over the biceps, and an increase in the amount of bone plus muscle At larger testis sizes the classical loss of fat occurred in the arm skinfolds whilst the subscapular skinfold underwent a gradual rise from the very beginning of puberty onward

In girls a loss of arm fat occurred initially which was followed by a steep rise late in puberty subscapular fat likewise rose during the latter stages of puberty The bone and muscle component remained unaltered at first and increased between breast development stages 2 and 3

It is concluded that the very first effects of sex hormones in the boy consist of accumulation of subcutaneous limb fat along with some muscle growth fat loss and more pronounced muscular development do not occur until the testes have reached a volume of over 5 ml It is curious to note that the boy with testes of 4 or 5 ml (i e clearly in puberty ) resembles the girl which has not yet started to come in puberty

These initial changes in boys have not before been reported in longitudinal studies One reason why they may have passed unnoticed is the fact that the pubescent boy passes very quickly through the various stages of testicular development Testes may grow from 3 ml to 10 ml within a period of 12 months with an interval of three months between observations usually employed in longitudinal studies the short phase during which the above changes occur may be missed

An initial rise in skinfold thickness has been repeatedly observed by the author in girls during treatment with androgens

J M Tanner & D Gupta (Institute of Child Health London, England) *A longitudinal study of the excretion of individual steroids during adolescence*

The urinary excretion of twenty-one individually estimated $C_{19}$ and $C_{1}$ steroids has been studied at 6 monthly intervals in 18 boys from 9 to 15 and 10 girls from 9 to 14 Growth, skeletal maturity and the development of secondary sex characteristics were followed simultaneously Separation and estimation of the steroids was done by sephadex extraction enzyme hydrolysis and solvolysis, one stage paper chromatography thin layer chromatography and gasliquid chromatography

There were marked and consistent differences between children in the excretion of most steroids before adolescence but some children who were high excretors before adolescence, of one steroid moved to be relatively low excretors in adolescence At adolescence there were rapid increases in the excretion particularly of testosterone, androsterone dehydroepiandrosterone and 11 $\beta$ hydroxyandrosterone in boys the chief rise was between stages 3 and 5 of the genitalia development (Androsterone from mean of 36 μg/kg/24 hr in stage 4 to 73 μg/kg/24 hr in stage 5 testosterone 0 37 μg/kg/24 hr in stage 3 0 49 μg/kg/24 hr in stage 4 and 0 57 in stage 5 ) The excretion of aetiocholanolone rose considerably less There was no rise in testosterone excretion in girls (0 16 μg/kg) and no rise in epitestosterone excretion in either sex Sex differences in excretion of these steroids became marked after age 12 Both age trends and sex differences were more pronounced when excretion was related to skeletal maturity than when related to chronological age No marked sex differences occurred in the excretion of cortisol metabolites during adolescence The glucuronide to sulphate ratio of the 11-deoxy 17 oxosteroids decreased consistently with increasing age

Z Laron & A Perzelan (Pediatric Metabolic and Endocrine Service Beilinson Hospital Univ Med School Tel Aviv Israel) *Clinical use of the antiandrogen cyproteron (SH-80 881) in the pediatric age group*

Cyproteron was administered in doses of 100–200 mg/day to 17 patients (14 females and 3 males) ranging in age from 6 ⁶/₁ to 18 ⁶/₁ years According to the diagnosis and indications for treatment they belonged to the following groups

I Precocious sexual development (5 pts)
II Cong adrenal virilism (6 pts)
III Cong adrenal virilism post puberal (2 pts)
IV Increased growth or hirsutism

The period of treatment ranged from 4 to 13 months

The results can be summarized as follows The amount and degree of *acne* was reduced in all patients to whom they were disturbing *Hirsutism* was difficult to evaluate *Sexual drive* as evidenced by masturbation was stopped *Menstruation* was not affected nor was body weight *Linear growth* was not significantly influenced however there was a tendency for decrease in growth velocity *Skeletal maturation* was unchanged but considering that most patients had an advanced bone age it is possible that maturation was slowed

There was no change in the daily excretion of the urinary 17KS but there was a marked increase in the total urinary 17 OHCS without clinical signs of hypercorticism An exception were two patients who developed striae after one year of continuous treatment They had no moon face or elevated blood pressure It is assumed that most of the total 17 OHCS are metabolites of the drug administered

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way in the most common form of congenital adrenal hyperplasia (CAH) A new method for the determination of the free 17-alpha hydroxy progesterone in plasma in the nanogram range was developed

The method includes the following steps Ether extraction, columnchromatography first thin layer chromatography (TLC) trifluoracetylation, second TLC, gas liquid chromatography (GLC) with an electron capture detector Correction for losses by an intern $^3$H 17 alpha hydroxyprogesterone standard As little as 0 5 ng of 17 alpha hydroxyprogesterone trifluoracetate is detectable by the GLC

The following values for 17 alpha hydroxy progesterone in plasma were found

| | Number of determinations (n) | Range (ng/100 ml) | Median value (ng/100 ml) |
|---|---|---|---|
| Normal children and adults | 54 | 0–860 | 150 |
| Children with treated CAH | 12 | 200–1500 | 700 |
| Children with untreated CAH | 8 | 4000–27 000 | 17 500 |
| Heterozygote parents of a child with CAH | 6 | 0–195 | 105 |

Precision of the method Variation coefficient $v=(\sigma \ 100)/\bar{x}$

Range $x < 1000$ ng/100 ml, $v=27$
Range $x > 1000$ ng/100 ml $v=8\ 7$

The method is suitable for the determination of 17-alpha hydroxyprogesterone plasma level in CAH There is no statistic significant difference between normal and heterozygote individuals But the relatively high values for normals are estimations from the beginning of this work and might therefore perhaps not be as accurate as the values determined later on

M Friedman (M R C Clinical Research Centre, Northwick Park, and University College Hospital, London, England) *A test of adrenocortical sensitivity in man Its application to bioassay of ACTH and its use in the assessment of possible altered adrenocortical sensitivity*

The administration of pharmacological quantities of adrenocorticotrophin (ACTH) followed by the measurement of plasma or urinary steroid levels is the basis of all currently used tests of adrenal function A test based on the administration of physiological amounts (nanogram quantities) of synthetic ACTH compounds has been devised to test adrenal sensitivity (Landon James, Wharton & Friedman, 1968) This procedure has proved to be valuable for assaying corticotrophin activity in man and for assessing adrenocortical sensitivity in children receiving prolonged ACTH therapy

A recently synthesized analogue of corticotrophin the penta cosapeptide D serine[1] norleucine[4] valinamide[25] B[1 25] corticotrophin (D W 75 Sandoz) was found to have an activity of 625 i u /mg when assayed by the rat adrenal ascorbic acid depletion test of Sayers The assay value obtained by this compound using the Sayers test was five times that obtained for synthetic porcine corticotrophin and the tetra cosapeptide synacthen (Ciba) D W 75 has been administered to human subjects in pharmacological and physiological concentrations and the adrenal response was measured The results indicate that on a weight for weight basis D W 75 has similar duration of action and adrenal stimulating properties to other synthetic polypeptides with adrenocorticotrophin action These findings suggest that the assay values based on adrenal ascorbic acid depletion test obtained with polypeptides having corticotrophin like activity bear little relationship to the behaviour of these preparations when administered to man

Adrenocortical sensitivity was assessed in a group of children who had been treated with ACTH for prolonged periods of time because of the possibility of altered adrenocortical responsiveness as a result of repeated stimulation The results indicate neither increased nor

b decreased adrenocortical sensitivity as a result of prolonged adrenal stimulation with exogenous ACTH

hyperaldosteronism could also be involved in the hypertension of other forms of childhood nephritis

Maria I New & J H Laragh (Cornell Univ Med Col and Columbia Univ Col of Physicians and Surgeons New York City N Y USA intr by W Swoboda) *Childhood hypertension associated with bilateral endocrine dysfunction of the kidney*

Two girls 9 and 11 manifested acute severe hypertension and encephalopathy Clinical studies revealed mild hypokalemia and marked oversecretion of aldosterone The aldosteronism was partially responsive to changes in dietary sodium and was not modified by dexamethasone Renin levels were equally elevated in both renal veins and in peripheral blood Indices of renal function—urinary protein and sediment creatinine clearance blood urea nitrogen and urine cultures—were repeatedly normal Renal biopsies revealed focal glomerulonephritis with normal to hyperplastic juxtaglomerular cells The data suggest that this hypertensive disorder with hyperreninemia and secondary hyperaldosteronism may be consequent to a focal glomerulonephritis in which the kidney acts as an abnormal endocrine organ while maintaining normal excretory function In both patients improvement in hypertension hyperreninemia and hyperaldosteronism occurred over one year This endocrine disorder characterized by bilateral renal parenchymal disease should be distinguished from (1) unilateral renal artery stenosis associated with hyperreninemia and hyperaldosteronism and from (2) other forms of primary adrenal cortical hypersecretion with hyperaldosteronism and depressed plasma renin Certain patients previously called congenital hyperaldosteronism in which renin was not measured could also be examples of this form of nephritis with secondary hyperaldosteronism [illegible] of renin with secondary

R P Zurbrugg (University Children's Hospital Bern Switzerland intr by A Schwenk) *Plasmocortisol rhythms in CNS-disorders*

Biologic rhythms are a basic characteristic of life among the most impressive periodic changes known are the daily oscillations of plasma cortisol

In the adult daytime activity parallels a diurnal decrease in plasma cortisol levels which is followed by a nocturnal rise This oscillation is characterized by both a frequency of about 24 hours and a distinct amplitude These characteristics of rhythmic changes such as frequency and amplitude may not only be influenced by age and environment but also by certain states of disease

We could already report that during infancy plasma cortisol rhythms with about two oscillations per day are found compared to the one cycle periodicity observed in older children and adults We could also demonstrate that changes in the environment lead to an increased frequency in plasma cortisol rhythms This observation is comparable to the above mentioned finding in early life time periods

With this as a background we have recently investigated diurnal cortisol rhythms in various CNS-disorders compared to normals

*1* In individuals with intracerebral organic lesions such as pontine tumor diencephalic syndrome internal hydrocephalus craniopharyngeoma and postcommotional syndrome we have found an increased frequency of cortisol oscillations as the common denominator in all these patients Again a finding which is comparable to the observations in early life time periods

On the other hand a normal frequency of about 24 hours has been found in apparently non-organic CNS-disorders i e where the

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pairment of proteolysis constitutes the main ef fect of KI (potassium iodide) in the toxic gland Although the euthyroid patient showed the same temporary block in organification the impairment of proteolysis was only minimal and delayed

J Girard & M Vest (Children s Hospital Universitatskinderklinik Basel Switzerland) *Evaluation of hypothalamo-pituitary function in children of short stature response of plasma glucose FFA cortisol and growth hormone to insulin induced hypoglycemia*

Insulinhypoglycemia was induced in 20 children of short stature of nonpituitary origin and in hypopituitary dwarfs by i v injection of 0 1 iU of insulin per kg body weight Plasma sugar free fatty acid cortisol and growth hormone (and in a few cases plasma ACTH) was measured before and at 20 minutes intervals after injection of insulin

The changes in plasma values are intercorrelated The definition of a normal response and the usefulness of insulininduced hypoglycemia as a test of pituitary function in children are discussed

H Helge & B Weber (Department of Pediatrics Free University of Berlin Germany) *Plasma insulin response to a 15 minute arginine-infusion in normal and obese children*

*In obesity adaptive hyperinsulinism is a well known phenomenon* Obese adults and children show hypersecretion of insulin after stimulation with glucose tolbutamide or glucagon Arginine however another stimulus of insulin release in healthy adults and children did not cause increased insulin response in a group of obese adults (1) In our studies plasma insulin levels have been compared in normal diabetic and obese children following an intravenous infusion of arginine (0 5 g per kg in 15 min) Mean insulin concentrations in the controls ($n=17$) had increased from 13 to 47 $\mu$U per ml 5 minutes after the infusion was ended In untreated diabetic children the insulin values did not respond at all and in the obese group ($n=5$ >20 overweight) a rise from a mean fasting level of 30 to a maximum of 204 $\mu$U per ml was observed These results differ from those of Copinschi *et al* in adults In obese children hyperinsulinism can apparently be demonstrated with arginine as well as with other stimuli

*Reference*

1 Copinschi G Wegienka L C Hane S & Forsham P H Effect of arginine on serum levels of insulin and growth hormone in obese subjects Metabolism *16* 485 196

H Lestradet & J Tichet (Centre d etudes sur le Diabete et la Nutrition chez l infant Hopital Herold Paris France) *Circadian variation in plasma insulin level*

deep cerebral structures of the brain stem have not been affected This was the case, e g in subjects with idiopathic precocious puberty, subdural effusion and anorexia nervosa

Therefore in relation to frequency the patients examined can be separated as individuals with organic CNS lesions associated with an increased frequency on the one hand and into patients with non organic CNS disorders associated with a normal frequency on the other hand

2 Special attention was paid to the investigation of plasma cortisol rhythms in dwarfism

A normal amplitude has been found in primordial dwarfism (both in so called low normals in Tanner s terminology and in patients with low birth weight dwarfism) Patients with hypopituitarism have been classified into four groups there the amplitude fell within normal limits only in one group namely in subjects with hereditary isolated growth hormone deficiency

However a small amplitude only could be demonstrated in all the remaining groups of hypopituitarism i e in craniopharyngeoma in patients with a positive history for birth injury and in so called idiopathic hypopituitary dwarfs of unknown etiology suggesting that they too may be associated with an organic CNS lesion most probably with a non traceable birth injury

In dwarfism then primordial dwarfs and children with hereditary isolated and therefore also non organic growth hormone deficiency only have a normal amplitude This is in contrast to the small amplitude found in the remaining groups of hypopituitarism with an organic etiology

In summary characteristics of plasma cortisol oscillations such as frequency and/or amplitude may not only be influenced by age and environment but also by certain organic CNS disorders This observation may not only give further physiopathological information but may also be of diagnostic importance

U K Buhler & Leslie J De Groot (Children's Hospital Basel, Switzerland and The Clinical Research Center, MIT, Cambridge Mass USA, intr by G Stalder) *The effect of large doses of stable iodine on the hyperthyroid gland*

Investigations on the action of large doses of stable iodine in hyperthyroid patients were performed Three toxic and one euthyroid patient received 75 mg potassium iodide daily their glands being prelabeled with $^{1\,2}$I 7–10 days before A second label of $^{131}$I was given 11–15 hours prior to the administration of KI Daily thyroid radioactivity labeled serum PBI and $^{1\,7}$BEI and urinary excretion of isotopes were determined over a 3–4 weeks period Within 24 hours after the administration of stable iodine there was a uniform decrease of $^{131}$I neck activity and a rise in urinary label excretion Within 2 days a maximum of urinary label excretion was obtained whereafter a constant decrease of urinary $^{131}$I excretion was noted Simultaneously the loss of neck activity was slowed down The pattern of $^{1}$ I label in the urine was the same but less marked Changes in thyroid $^{1}$ I activity were minimal in two cases thus no change in the slope could be demonstrated In the pretreatment phase the slope of the neck radioactivity and that of the urinary label excretion are parallel After 2 days of KI treatment these two slopes are no longer parallel The urinary label excretion decreases faster than the loss of radioactivity over the neck While on KI there was a constant drop of $^{1\,7}$BEI This decrease of hormone was detected already after 24 hours The parallel decrease of $^{1\,7}$BEI and $^{1\,8}$PBI strongly suggests that there is no isotope dilution

We interpreted our results as follows The stable iodine produces an early block in organification of iodine This block is partially overcome after 2 days Simultaneously there is a constant impairment of proteolysis of all iodotyrosins and iodothyronins as seen in the reduction of circulating hormone and in the decrease of urinary label excretion This im

pairment of proteolysis constitutes the main effect of KI (potassium iodide) in the toxic gland. Although the euthyroid patient showed the same temporary block in organification the impairment of proteolysis was only minimal and delayed.

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A normal amplitude has been found in primordial dwarfism (both in so called low normals in Tanner s terminology and in patients with low birth weight dwarfism) Patients with hypopituitarism have been classified into four groups there the amplitude fell within normal limits only in one group namely in subjects with hereditary isolated growth hormone deficiency

However a small amplitude only could be demonstrated in all the remaining groups of hypopituitarism i e in craniopharyngeoma in patients with a positive history for birth injury and in so-called idiopathic hypopituitary dwarfs of unknown etiology suggesting that they too may be associated with an organic CNS lesion most probably with a non traceable birth injury

In dwarfism then primordial dwarfs and children with hereditary isolated, and therefore also non organic growth hormone deficiency only have a normal amplitude This is in contrast to the small amplitude found in the remaining groups of hypopituitarism with an organic etiology

In summary characteristics of plasma cortisol oscillations such as frequency and/or amplitude may not only be influenced by age and environment but also by certain organic CNS-disorders This observation may not only give further physiopathological information but may also be of diagnostic importance

U K Buhler & Leslie J De Groot (Children's Hospital Basel Switzerland and The Chemical Research Center MIT Cambridge Mass. USA intr by G Stalder) *The effect of large doses of stable iodine on the hyperthyroid gland*

Investigations on the action of large doses of stable iodine in hyperthyroid patients were performed Three toxic and one euthyroid patient received 75 mg potassium iodide daily their glands being prelabeled with $^{125}$I 7–10 days before A second label of $^{131}$I was given 11 15 hours prior to the administration of KI Daily thyroid radioactivity labeled serum PBI and $^{127}$BEI and urinary excretion of isotopes were determined over a 3–4 weeks period Within 24 hours after the administration of stable iodine there was a uniform decrease of $^{131}$I neck activity and a rise in urinary label excretion Within 2 days a maximum of urinary label excretion was obtained whereafter a constant decrease of urinary $^{131}$I excretion was noted Simultaneously the loss of neck activity was slowed down The pattern of $^{1}$ I label in the urine was the same but less marked Changes in thyroid $^{125}$I activity were minimal in two cases thus no change in the slope could be demonstrated In the pretreatment phase the slope of the neck radioactivity and that of the urinary label excretion are parallel After 2 days of KI treatment these two slopes are no longer parallel The urinary label excretion decreases faster than the loss of radioactivity over the neck While on KI there was a constant drop of $^{127}$BEI This decrease of hormone was detected already after 24 hours The parallel decrease of $^{1}$ BEI and $^{125}$PBI strongly suggests that there is no isotope dilution

We interpreted our results as follows The stable iodine produces an early block in organification of iodine This block is partially overcome after 2 days Simultaneously there is a constant impairment of proteolysis of all iodotyrosins and iodothyronins as seen in the reduction of circulating hormone and in the decrease of urinary label excretion This im

pairment of proteolysis constitutes the main effect of KI (potassium iodide) in the toxic gland. Although the euthyroid patient showed the same temporary block in organification the impairment of proteolysis was only minimal and delayed.

J. Girard & M. Vest (Children's Hospital, Universitätskinderklinik, Basel, Switzerland): *Evaluation of hypothalamo-pituitary function in children of short stature: response of plasma glucose, FFA, cortisol and growth hormone to insulin induced hypoglycemia.*

Insulinhypoglycemia was induced in 20 children of short stature of nonpituitary origin and in hypopituitary dwarfs by i.v. injection of 0.1 IU of insulin per kg body weight. Plasma sugar, free fatty acid, cortisol and growth hormone (and in a few cases plasma ACTH) was measured before and at 20 minutes intervals after injection of insulin.

The changes in plasma values are intercorrelated. The definition of a normal response and the usefulness of insulininduced hypoglycemia as a test of pituitary function in children are discussed.

H. Helge & B. Weber (Department of Pediatrics, Free University of Berlin, Germany): *Plasma insulin response to a 15 minute arginine-infusion in normal and obese children.*

In obesity adaptive hyperinsulinism is a well known phenomenon. Obese adults and children show hypersecretion of insulin after stimulation with glucose, tolbutamide or glucagon. Arginine, however, another stimulus of insulin release in healthy adults and children, did not cause increased insulin response in a group of obese adults (1). In our studies plasma insulin levels have been compared in normal, diabetic and obese children following an intravenous infusion of arginine (0.5 g per kg in 15 min). Mean insulin concentrations in the controls ($n=17$) had increased from 13 to 47 $\mu$U per ml 5 minutes after the infusion was ended. In untreated diabetic children the insulin values did not respond at all, and in the obese group ($n=5$, $>20\%$ overweight) a rise from a mean fasting level of 30 to a maximum of 204 $\mu$U per ml was observed. These results differ from those of Copinschi *et al.* in adults. In obese children hyperinsulinism can apparently be demonstrated with arginine as well as with other stimuli.

*Reference*


1. Copinschi, G., Wegienka, L. C., Hane, S. & Forsham, P. H.: Effect of arginine on serum levels of insulin and growth hormone in obese subjects. *Metabolism 16*: 485, 1967.


H. Lestradet & J. Tichet (Centre d'etudes sur le Diabete et la Nutrition chez l'infant, Hopital Herold, Paris, France): *Circadian variation in plasma insulin level.*

# PROCEEDINGS OF PEDIATRIC SOCIETIES

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A series of biopsies from the oral mucosa was examined histologically The material consisted of 22 biopsies from 11 children with the clinical diagnosis mucoviscidosis from 6 children with suspected mucoviscidosis and from 5 controls Thirty sections from each biopsy were microscoped under code number

The following histological criteria were used Slight alterations consisting of duct dilation flattening of the ductal epithelium, and inspissated eosinophilic material in the ductal lumina and marked alterations consisting of the above mentioned findings plus inspissated material in the acini atrophy of the acini and possibly fibrosis

From the group with clinically verified mucoviscidosis three biopsies showed marked alterations and five showed slight alterations Thus 72 per cent were abnormal In the group with clinically suspected mucoviscidosis one showed marked and two slight alterations, i e 50 per cent abnormal In the control material no alterations were found

Our results are in accordance with the find

ings in the larger series published by Warwick *et al* (1) that consisted of 25 patients 80 per cent showed abnormalities and that of Sweney *et al* (2) consisting of 28 patients with abnormal biopsies in 82 per cent.

There was a considerable difference in the histological criteria in the two works In the first work ten criteria were used whereas in the later publication only the finding of eosinophilic plugs was used In our series we have also considered atrophic acini as being diagnostic because they are similar to the alterations in the pancreas

It is concluded that the histological method may confirm the diagnosis of mucoviscidosis in most cases but that it cannot replace the sweat test It may be useful in some situations with uncertain diagnosis

*References*


1 Warwick W J Bernard B & Meskin L H The involvement of the labial mucous salivary gland in patients with cystic fibrosis *Pediatrics* *34* 6 1-678 1964
2 Sweney L R Hedrick M C Meskin L H & Warwick W J The involvement of the labial mucous salivary gland in patients with cystic fibrosis II The heterozygote state *Pediat scr* *40* 471-4 4 1967


Paul S Symchych John Wanstrup and Vagn Andersen (Copenhagen) *Chronic granulomatous disease of childhood*

The pathological findings in three patients with Chronic Granulomatous Disease (CGD) are presented Special emphasis is placed on the pathognomonic histological combination of pigmented macrophages and granuloma formation The macrophages which contain a yellow sudanophilic PAS positive pigment are found dispersed in the tissues Their presence in the submucosa and lamina propria of the large intestine suggests that rectal biopsy may be of help in the diagnosis of CGD

Unimpaired phagocytosis, but defective bactericidal capacity of the neutrophil granulocytes has been demonstrated in this disease Evidence is presented that the other phagocytic cells of these patients do show at least some degree of normal function

Stig Ramstrom (Goteborg) *On the pathogenesis of chronic subdural hematoma*

It is a wellknown fact that an acute subdural hemorrhage is resorbed by a membrane of granulation tissue formed on the inner surface of the dura It was demonstrated that this granulation membrane becomes very loosely attached to the dura because of an edematous spongiosity occurring in the part of the membrane next to the original inside surface of the dura It may be supposed that larger accumulations of fluid within this part of the membrane may result in hygroma of the dura It was shown that small bleedings occur within the spongious part of the membrane If there is a more extensive accumulation of blood this will have the character of an encapsulated subdural hematoma (= pachymeningitis haemorrhagica interna) Thus the chronic encapsulated subdural hematoma is considered to be a bleeding within the granulation membrane formed on the inner surface of the dura in order to resorb an acute free subdural hemorrhage (whatever the cause of this primary bleeding may be) Macro- and microscopic pictures supporting this view were demonstrated

Bengt Larsson (Borås) *Unrecognized tumour of the heart causing sudden death at the age of four months*

A baby boy who had previously shown slight signs of asthmatic bronchitis died unexpectedly during nursing. Autopsy revealed a well developed infant with no sign of aspiration but with subacute edema patchy atelectase of the lungs and venous congestion The heart was enlarged weighing 145 g, including a white dense tumour resembling an uterine fibroid and weighing 100 g The tumour was slightly bosselated and involved the posterior lateral and septoapical portions of the left ventricle The tumour was moderately vascular

*Acta Pædiat Scand 58 208–212, 1969*

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-bolition of all clinical signs of hypothyroidism and a rise in the PBI level At a time when the serum concentration of total iodide was 11 4 μg per 100 ml the PBI level was 5 4 μg per 100 ml and the BEI level 4 0 μg per 100 ml

The clinical and pathologic findings as well as the results of the thyroid function studies in these patients suggest a defect in the iodide trapping mechanism of the thyroid gland This assumption was substantiated by the fact that high serum concentrations of inorganic iodide apparently compensated for this defect and restored hormone synthesis possibly by forcing sufficient iodide into the gland by mass action However since the salivary glands showed some ability to concentrate ¹³¹I it is presumed that the trapping defect was only partial Defective iodide trapping mechanism is the least frequent form of goitrous hypothyroidism due to inborn defects in the synthesis of thyroid hormones Until now only 4 such cases have been reported Further studies on the present cases are in progress and will be reported elsewhere

Ernest Gluck (Bergen) *Congenital hypothyroidism Histological considerations*

In two sisters with a possible iodine trapping defect presented by Aarskog & Runde there were multiple and in one case bilateral adenomas in the thyroid The histological picture was almost identical showing multiple trabecular and microfollicular areas with little colloid The surrounding thyroid tissue showed intense and in places infiltrative proliferation with prominent nuclear monstrosities The possibility of malignancy was considered in one of the patients

On reviewing our thyroid material we found this highly characteristic histological picture in two further patients

1 Fifteen year-old girl Treated for hypothyroidism since the age of 7 months A goitre (310 gm) compressed the trachea with increasing respiratory distress The diagnosis of carcinoma was discussed here too The later course has not supported that diagnosis

2 Another patient a boy was operated for recurring thyroid adenomas at 7 9 and 17 years of age Hypothyroidism was not considered at first as both basal metabolic rate and mental function were normal The cholesterol values were however raised (350 mg) His growth was below average for his age and he had a hypochromic anaemia He has been given thyroid treatment and no further adenomas have developed Investigations for a possible enzyme defect in thyroxin synthesis were not carried out on these patients

The histological picture described is as has been stressed also in the literature on congenital hypothyroidism extremely characteristic in these cases including some with enzyme defect This picture is suggestive of a defect in thyroid function even if the usual clinical criteria for hypothyroidism are not or only slightly in evidence

In spite of the histological signs of malignancy the prognosis is in our experience good being dependent on adequate substitution therapy

Knut Jacobsen (Bergen) *Giant cell pneumonia*

An eleven year-old boy was admitted to hospital in March 1968 with respiratory symptoms He was the eldest of five and had previously been well apart from recurrent respiratory infections over the past few months His appetite was bad and he had lost weight He had been exposed to measles 14 days before admission For the past week he had had a high fever with weakness and increasing dyspnoea On examination he was found to have bilateral pneumonia and X ray examination showed scattered infiltrates throughout both lungs Nine hours after admission his dyspnoea increased suddenly and he died Clinical diagnosis virus pneumonia

At autopsy the lungs were found to be hyperaemic smooth and shiny On section the surface consisted of relatively firm yellowish grey infiltrates of from 1 mm to 1 cm in dia-

and consisted of collagenous fibres and a fair number of elastic fibrils These formed both a dense lamellar and a looser whirly pattern The former had few nuclei and gave in part the im pression of a petrified myocardium The latter pattern was rich in nuclei being made up of primitive connective tissue It contained cell groups interpreted as extramedullary haemopoiesis The fibroelastic tissue intermingled with muscle bundles of varying calibre It was hard to exclude striated muscle as a possible precursor of the fibroelastic mass

The tumour resembled the intramural fibroma of the heart, described by Clay & Shorter (1957) Biegelow *et al* (1954) proposed the term primitive fibroma or rhabdomyofibroma believing the parenchyma to be a genuine component of the tumour tissue In 1956 Conlon coined the term embryonic me senchymal tumour of the heart Willis (1958) deals with the present tumour under the heading of infantile and juvenile fibromas fibromatosis and fibromatoid lesions

The demonstrated lesion represents a mus culoaponeurotic or even sarcolemmal maldevelopment coming close to the so called desmoid tumours Possibly the intramural fibroma represents one extreme of a maldevelopmental process where the so called rhabdomyoma with spider cells represents the other extreme but other developmental lesions usually accompany the latter form

Bengt Robertson (Stockholm) *Postnatal for mation and obliteration of arterial bronchopul monary anastomoses*

Microangiographic and histologic studies on the incidence and structure of arterial broncho pulmonary anastomoses in infancy and early childhood revealed that the number of anastomoses increases with postnatal age The op posite holds for the pulmobronchial arteries (intrapulmonary bronchial arteries originating from the pulmonary artery) and it seems prob able that some anastomoses are formed from pulmobronchial arteries which establish precapillary communication with adjacent branches of true bronchial arteries Intimal bundles of smooth muscle cells ( Sperr artery structure) were found in many anastomoses, from the age of 2 ¹/ months Anastomoses which were completely obliterated by intimal smooth muscle cells and fibrous tissue were observed from the age of 7 months Most of the arterial bronchopulmonary anastomoses probably become obliterated towards normal adult age

Dagfinn Aarskog (Bergen) & Ingvar Rode (Oslo) *Familial hypothyroid nodular goiter with possible iodide trapping defect*

Two sisters presented with nodular goiter and clinical signs of hypothyroidism at the age of 7 to 8 years There was no intermarriage in the family and no known cases of thyroid dis order Both girls had low serum levels of PBI 0 5 μg per 100 ml and 1 5 μg per 100 ml respectively and elevated serum cholesterol 324 mg per 100 ml and 526 mg per 100 ml respectively In one of them there was virtually no radio iodine uptake in the thyroid gland 24 hours after an oral dose of 60 μc of $^{131}$I In the other patient only a small fraction of the administered $^{131}$I accumulated in the gland The uptake after 4 hours was 4 per cent and after 24 hours 3 per cent The uptake remained unchanged following stimulation with TSH and there was no release of radioactivity fol lowing administration of perchlorate The renal iodine clearance was normal Chromatography of urine obtained during the first 6 hours after intravenous administration of $^{131}$I labelled mono iodotyrosine (MIT) did not reveal any radio iodine tagged MIT in the urine An in ability of the thyroid to trap iodine was sus pected Since the ability to concentrate iodine is shared by the thyroid and the salivary glands the trapping mechanism can be studied determination of the salivary $^{131}$I to plasma $^{131}$I ratio after ingestion of $^{131}$I The fol lowing ratios were found 8 9–9 2–13 1 and 9 8 (2 4 6 and 8 hours after $^{131}$I inges tion) These ratios were considered subnormal A therapeutic trial with potassium iodine in a dose of 1 mg 3 times daily was followed by

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# FAMILIAL HYPOPHOSPHATEMIC VITAMIN D RESISTANT RICKETS

## The Neonatal Period and Infancy

GUNNAR B STICKLER

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The question whether growth failure can be prevented in patients with vitamin D resistant rickets (VDRR) by beginning treatment with high doses of vitamin D during the first 6 months of life has been debated recently Harrison and co-workers (8) did not demonstrate such an effect in three patients with this disease However Schoen (13 14) believed that normal growth has been maintained in one child with VDRR treated in such a fashion Furthermore Schoen (13) found in his patient hypophosphatemia in the neonatal period and throughout the observation period whereas this presumably was not noted by Harrison and his group (8)

In an attempt to get further information about the early biochemical changes and growth patterns in patients with VDRR 16 investigators interested in the field were contacted This report comprises an analysis of the early biochemical findings and growth during the first few years of life of the five infants whose cases were previously reported and some data on three additional patients with VDRR including one from the Mayo Clinic Also included are growth data on 10 patients with VDRR who had been seen at the Mayo Clinic during the first 5 years of life before and after treatment with high doses of vitamin D

Analysis of data shows that hypophosphatemia is present in the neonatal period in patients with VDRR that alkaline phosphatase is elevated at 1 month of age and that early treatment with high doses of vitamin D does not seem to prevent growth failure whenever this treatment is begun

## MATERIAL AND METHODS

The patients included in this analysis fulfilled the criteria of VDRR of the familial hypophosphatemic type as outlined by Williams and co workers (18) One patient (case 1) has been described by Tapia et al (16) More detailed data regarding the height measurement of this patient were supplied by Dr Genevieve Stearns Information on four patients reported by Harrison and associates (8) (cases 2 3 4 and 5) one patient (case 6) by Schoen (13) and the three additional patients (cases 7 8 and 9) is included in this report

Some clinical data of the patients observed during the first year of life are shown in Table 1 Details about the clinical course in the previously reported cases can be found in the original publications The clinical history in the heretofore unpublished cases is briefly summarized

## CASE REPORTS

*Case 7*

The mother of this patient had been treated for VDRR since the age of 3 years with doses of vitamin D between 30 000 and 50 000 units per day She had severe bowing of her legs She had one daughter with this disease The mother had her second pregnancy when she was 30 years old During the pregnancy she was initially treated with a daily dose of 50 000 units of vitamin D this dose was decreased to 35 000 units per day during the latter part of her pregnancy

meter Microscopy showed multinuclear giant cells of foreign body type lying mainly in the alveolar spaces There were no inclusions There were small reaction centres in the lymphatic tissue but no giant cells There was no measles antibody in the serum post mortem and no virus was found in tissue from the lungs

The patient's 4 year younger brother was admitted the following day with similar symptoms, although he had an atypical rash on both sides of the thorax He too had been exposed to measles This patient survived and was found to have plenty of antibody 4 months later He too had had frequent respiratory infections during the previous winter Four to 5 days after these two had been admitted to hospital the three youngest children developed measles with characteristic symptoms and course

We feel that it is extremely likely that the cause of death in this eleven year-old boy was respiratory insufficiency due to a giant cell pneumonia caused by the measles virus The lack of antibody production can not be explained It has been suggested that it may be due to a general depression of the reticulo-endothelial system brought about by previous longstanding recurrent infections

Jon Lamvik (Bergen) *Lymph node changes following antigen stimulation*

A survey was given of experimental data found in recent medical literature about the changes in lymphoid organs which follow differ[illegible] types of antigen stimulation

1 After stimulation with antigens wh[illegible] give delayed allergic reaction without serum [illegible] antibody production increased numbers [illegible] large pyroninophilic cells with signs of c[illegible] proliferation are found in the deep cortic[illegible] (paracortical) layer without germinal cente[illegible] in the follicles and without plasma cell form[illegible] tion

2 In the allograft reaction similar lymp[illegible] node changes may be observed but in additio[illegible] to the paracortical cell reaction enlarged ge[illegible] minal centers and plasma cells in the medull[illegible] may be found

3 Following injection of antigens whic[illegible] cause antibody production enlargement of ge[illegible] minal centers with cell proliferation has bee[illegible] observed There is evidence of cell migratio[illegible] from the germinal centers to the surroundin[illegible] lymphoid tissue and concomitant appearanc[illegible] of large pyroninophilic cells and plasma cel[illegible] containing antibodies in cortex and medulla

Thus, two types of lymph node changes ma[illegible] be induced by antigen stimulation, although [illegible] mixed reaction often predominates

The alterations observed in experiment[illegible] animals following stimulation with differen[illegible] types of antigen were used to try to explai[illegible] some of the changes observed in human lymp[illegible] node biopsies, showing so called reactive hy[illegible] perplasia

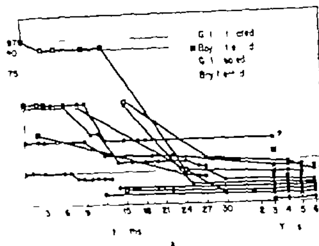


Fig 1 Growth failure in vitamin D resistant rickets

were 3 years old Two children were 4 years old and one child was 5 years old before treatment was begun

Control data for urinary creatinine calcium and inorganic phosphate were compiled from measurements in 10 normal male infants during a 24 hour period beginning on the second or the third day of life The infants were receiving an evaporated milk formula identical to the formula given to the patients with VDRR Levels of serum and urinary creatinine were determined by the Folin Wu procedure adapted to the AutoAnalyzer (17) serum calcium by the method of Jones & McGockin (9) and urinary calcium by use of the complexometric titration of Yarbo & Colby (19) Inorganic phosphate values were determined by use of the Fiske Subba Row procedure adapted for serum determination to the AutoAnalyzer by Logan and co workers (6) and urinary phosphate values were determined by use of the Gomori modification (7)

The control data for levels of serum inorganic phosphate for the newborn period were those collected by Brock & Weintraub (?) for the age period from 1 month to 6 months by Lomon (5) and for the age period from 7 to 12 months by Bullock (3) Data for the renal phosphate clearances in the newborn period had been collected by Richmond and associates (1 ) and McCrory and co-workers (10) Control data for the ratio of calcium to creatinine after the newborn period were calculated from the data by Daniel et al (4)

## RESULTS

### Growth

The growth data are summarized in Fig. 1 All heights were recorded by determining the height percentile from the anthropometric charts published by the Children's Medical Center in Boston The heights of almost all patients were below the third percentile by the age of 2 1/2 years with the exception of Schoen's patient (case 6) In his latest communication Schoen (14) mentioned only that the patient had normal height at age 3 years but he did not give the actual measurement However patient no 8 had remained in the 10th percentile although he was not treated until he was 19 months old Treatment does not seem to influence growth failure regardless of when it began Furthermore growth retardation begins at about age 7 to 12 months data suggestive of a correlation with the age of weight bearing

### *Biochemical findings*

*Serum phosphate* All serum determinations of inorganic serum phosphate obtained during the first 12 months of life in cases reported in the literature and in the three cases mentioned in this report (cases 7 8 and 9) are plotted in Fig. 2 The normal data are represented by means ±1 standard deviation for the first 6 months of life and as ranges for the ages from 7 to 12 months because less data were available for this age period The data show that all patients studied so far have hypophosphatemia even in the neonatal period Whether or not the mother had been treated with vitamin D

Table 1 *Clinical data on patients with familial hypophosphatemic vitamin D resistant rickets*

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| 6 | M | Mother | Yes | 50 | Schoen (13) |
| 7 | M | Mother | Yes | 48 | Stearns* |
| 8 | F | Mother | No | 51 | Finberg* |
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Personal communication to the author

The patient was born on June 5 1964 His birth weight was 3310 g and his height was 49 cm He appeared to be normal in all respects When he was 5 weeks old tests revealed a serum calcium level of 9 2 mg/100 ml a serum inorganic phosphate level of 4 3 mg P/100 ml and an alkaline phosphatase value of 19 J K units (normal 15 to 20 J K units at this age) Despite urging the mother did not return with the patient until he was 19 months old at which time *the boy was slightly bow legged and had active rickets as noted on roentgenographic examination*

*Case 8*

The mother as well as a maternal uncle of this patient had VDRR The mother was treated with high doses of vitamin D This patient was born by cesarean section because of pelvic abnormalities of *the mother presumably* due to the rickets Measurements during the neonatal period revealed a serum calcium level of 10 5 mg/100 ml an inorganic serum phosphate level of 5 8 mg P/100 ml and an alkaline phosphatase value of 10 King Armstrong units VDRR was diagnosed when the child was seen by Dr Laurence Finberg However accurate height measurements had been recorded by the family physician (Table 2) Treatment began when the patient was 19 months old

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The mother of this patient *had been treated for* VDRR since the age of 4 years She was short and had severe bowing of her legs During her pregnancy she had received a daily dose of 50 000 units of vitamin D Vaginal delivery of this patient *at one* institution was without *complications* His birth weight was 2800 g and he was 48 cm long No abnormalities were noted at the initial physical examination Roentgenographic examination *gave no* evidence that the patient had rickets The patient was given an evaporated milk formula and received 400 units of vitamin D as part of his supplementary vitamins Data concerning his growth are shown in *Fig 1* and biochemical data are recorded in Tables 2 and 3 When the patient was 3 months old because of rising values for alkaline phosphatase and early structural bone abnormalities the daily dose of vitamin D *was increased to 10 000 units per day* with further increase to 15 000 units at 5 months to 25 000 units at 9 months and to 30 500 units at 10 months The patient sat by himself at 7 months of age began to stand at 8 months and walked without support at 12 months Repeated roentgenographic examinations showed healing of the rickets

The files of the Mayo Clinic were reviewed in order to find patients with VDRR who were seen before they were 6 years old and who were not treated *before their first examination at our clinic Included* were patients with severe deformities as well as children with milder forms of VDRR who were seen earlier because the condition had been noted in one or more of their older siblings All these children had at least one parent or one or more of their siblings with VDRR After their initial visit and the establishment of the diagnosis they were treated with vitamin D in doses of from 50 000 to 75 000 units per day and their growth was observed Two of these children were seen before they were 1 year old three before they were 2 years old and two before they

Table 2 *Height and serum biochemical data in two cases (patients 8 and 9) with vitamin D resistant rickets*

| Age | Height (cm) Case 8 | Height (cm) Case 9 | Calcium (mg/100 ml) Case 8 | Calcium (mg/100 ml) Case 9 | Phosphate (mg P/100 ml) Case 8 | Phosphate (mg P/100 ml) Case 9 | Alkaline phosphatase (K A units) Case 8 | Alkaline phosphatase (K A units) Case 9 |
|---|---|---|---|---|---|---|---|---|
| Birth | 51 | 48 | 10 5 | | 5 8 | | 10 | |
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| 3rd day | | | | 8 5 | | 3 1 | | |
| 6 wk | | 54 | | 10 5 | | 4 0 | | 15 |
| 7 wk | 56 | | | | | | | |
| 3 mo | 58 | 58 | | 9 8 | | 3 3 | | 48 |
| 4 mo | | | | 10 1 | | 3 1 | | 75 |
| 5 mo | 64 | 62 5 | | 10 3 | | 4 0 | | 77 |
| 6 mo | 65 | 64 | | 10 0 | | 3 0 | | 51 |
| 7 mo | | 65 | | 10 4 | | 3 2 | | 58 |
| 8 mo | | 66 | | 10 4 | | 3 7 | | 67 |
| 9 mo | 70 | 67 | | 10 1 | | 3 7 | | 71 |
| 10 mo | | 68 | | 10 1 | | 3 0 | | 69 |
| 11 mo | 70 5 | 68 5 | | 10 4 | | 3 2 | | 61 |
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| 13 mo | 72 | 71 | | 10 0 | | 3 7 | | 54 |
| 14 mo | | | | | | | | |
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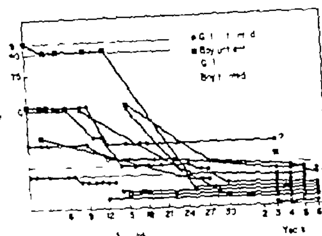


*Fig. 1.* Growth failure in vitamin D resistant rickets.

were 3 years old. Two children were 4 years old and one child was 5 years old before treatment was begun.

Control data for urinary creatinine, calcium and inorganic phosphate were compiled from measurements in 10 normal male infants during a 24-hour period beginning on the second or the third day of life. The infants were receiving an evaporated milk formula identical to the formula given to the patients with VDRR. Levels of serum and urinary creatinine were determined by the Folin-Wu procedure adapted to the AutoAnalyzer (17), serum calcium by the method of Jones & McGuckin (9), and urinary calcium by use of the complexometric titration of Yarbo & Golby (19). Inorganic phosphate values were determined by use of the Fiske-Subba Row procedure adapted for serum determination to the AutoAnalyzer by Frings and co-workers (6), and urinary phosphate values were determined by use of the Gomori modification (7).

The control data for levels of serum inorganic phosphate for the newborn period were those collected by Bruck & Weintraub (2), for the age period from 1 month to 6 months by Fomon (5), and for the age period from 7 to 1 months by Bullock (3). Data for the normal phosphate clearances in the newborn period had been collected by Richmond and associates (1 ) and McCrory and co-workers (10). Control data for the ratio of calcium to creatinine after the newborn period were calculated from the data by Daniels et al. (4).

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The growth data are summarized in Fig. 1. All heights were recorded by determining the height percentile from the anthropometric charts published by the Children's Medical Center in Boston. The heights of almost all patients were below the third percentile by the age of 2½ years with the exception of Schoen's patient (case 6). In his latest communication, Schoen (14) mentioned only that the patient had normal height at age 3 years but he did not give the actual measurement. However, patient no. 8 had remained in the 10th percentile although he was not treated until he was 19 months old. Treatment does not seem to influence growth failure, regardless of when it began. Furthermore, growth retardation begins at about age 7 to 12 months, data suggestive of a correlation with the age of weight bearing.

*Biochemical findings*

*Serum phosphate.* All serum determinations of inorganic serum phosphate obtained during the first 12 months of life in cases reported in the literature and in the three cases mentioned in this report (cases 7, 8, and 9) are plotted in Fig. 2. The normal data are represented by means ±1 standard deviation for the first 6 months of life and as ranges for the ages from 7 to 12 months because less data were available for this age period. The data show that all patients studied so far have hypophosphatemia even in the neonatal period. Whether or not the mother had been treated with vitamin D

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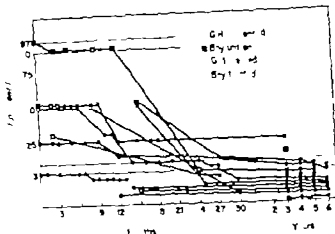


Fig. 1. Growth failure in vitamin D resistant rickets.

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[a] Bessy and Lowry units (normal up to 6 units)

Table 3 *Urinary calcium and phosphate excretion of one patient (case 9) compared to controls*

| | Before treatment | | After treatment was begun | |
|---|---|---|---|---|
| | Age 3 days | Age 3 mo | Age 4 mo | Age 6 mo |
| Calcium (mg/24 hr) | | | | |
| Patient | 0.2 | 12.8 | 8.0 | 3.6 |
| Control | (10 infants) | (4 infants) | (5 infants) | (2 infants) |
| Mean | 0.37 | 34 | 41 | 37[a] |
| Range | 0.005–1.0 | 15–43 | 27–48 | 20 and 43 |
| Ratio of cal um to creatinine | | | | |
| Patient | 0.0100 | | 0.089 | 0.083 |
| Control | (10 infants) | | (5 infants) | (2 infants) |
| Mean | 0.1 7 | | 0.495 | 0.251 |
| Range | 0.0021–0.4 | | 0.179 0.905 | 0.181–0.517 |
| Phosphate (mg P/24 hr) | | | | |
| Patient | 26 | 168 | 326 | 352 |
| Control | (10 infants) | (4 infants) | (5 infants) | (2 infants) |
| Mean | 5.5 | 348[a] | 356 | 311 |
| Range | 0.03 18.0 | 291 378 | 2 6–449 | 326 and 376 |
| Ratio of phosphate to creatinine | | | | |
| Patient | 1.3 | | 3.26 | 7.65 |
| Control | (10 infants) | | (5 infants) | (2 infants) |
| Mean | 0.198 | | 4.47[a] | 4.33 |
| Range | 0.002–0.563 | | 3.36–4.94 | 4.13–4.53 |
| Phosphate clearance (mg P/24 hr) | | | | |
| Patient | 6.28 | 77.8 | 55.0 | 43.8 |
| Control | (7 infants)[b] | (1 infant) | | (1 infant) |
| Mean | 7.1 and 7.7 | 30.4[c] | | 13.6[c] |
| Range | 37 11.6 | | | |

[a] Data calculated from Dancis and associates (4)
[b] Data calculated from Richmond and associates (12)
[c] Data calculated from McCrory and associates (10)

## COMMENT

Most of these patients who were followed up since early infancy were observed in different clinics and certain variations in the method of observation no doubt exist However height measurements obtained by different observers probably do not vary significantly and would not account for the trend in growth characteristics observed in infants and children with VDRR The determinations for inorganic serum phosphate also have become sufficiently standardized to allow conclusions even when determinations are carried out in different institutions

Data concerning the growth of infants with VDRR are not dissimilar from those obtained in older children Schoen s patient is a notable exception showing normal growth until the age of 3 years This patient had severe hypercalcemia at ages 8 and 9 months at a time when growth failure usually becomes apparent

Harrison and co-workers (8) have described one patient with VDRR who had significant vitamin D intoxication with reduced glomerular filtration rate presumably causing the level of serum inorganic phosphate to remain within normal limits and therefore preventing growth failure This does not seem to be true of Schoen s patient who had no increase in the level of inorganic serum phosphate during the period of hypercalcemia

It may be argued that Schoen s patient received sufficient doses of vitamin D to assure proper growth whereas the patient described in detail in this report and those treated by Harrison and co-workers did not However the alkaline phosphatase levels in patients 6 and 9 seem to parallel each other well with the exception of the period during which hypercalcemia was noted in patient 6 (Fig 3) It seems more likely that a variation of the natural disease process was observed by Schoen Re

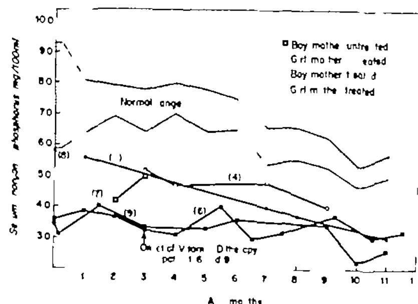



*Fig 2* Serum inorganic phosphate (mg P/100 ml) levels in patients with VDRR during the first 12 months of life Normal range is plotted for the first six months of life as mean ±1 SD and as ranges for the ages 7 to 12 months

during the pregnancy does not seem to make any difference in the levels of serum inorganic phosphate The onset of vitamin D therapy in the affected children did not alter the level of serum inorganic phosphate (Fig 2)

Serial determinations of alkaline phosphatase were made in two patients (cases 6 and 9) during the first year of life A single determination was made in the neonatal period in one patient (case 7) Elevations were observed at ages 1 and 3 months respectively The values in the neonatal period were normal in three patients who had been studied but the mothers of these three patients had been treated with high doses of vitamin D Definite elevation of alkaline phosphatase level occurred at ages 1 month and 3 months and a lowering of the blood levels occurred after vitamin D therapy in high doses was begun During a period of hypercalcemia due to excessive doses of vitamin D in one patient (case 6) at ages 8 and 9 months, the serum calcium levels were 14 6 and 11 mg respectively whereas the serum alkaline phosphatase level decreased significantly No corresponding change in growth rate was detectable in the graph supplied by Schoen (13) who had studied this patient

*Urinary calcium and phosphate excretion* Data are available on these factors only for one patient (case 9) The question whether this patient had hypocalcemia and hypophosphaturia with an increased phosphate clearance (due to the lowered serum phosphate concentration) in the neonatal period was of particular interest since this is the usual situation in older children with VDRR (15) The values for urinary calcium and phosphate excretion of this patient (case 9) as compared with those of the controls are shown in Table 3

Without measurements of the accurate intake of calcium only tentative conclusions from these data can be drawn Nordin (11) has pointed out that the ratio of calcium to creatinine is independent of calcium intake in adults but this hypothesis has never been tested in infants However phosphate clearances should be independent of phosphate intake and the patient's phosphate clearance in the neonatal period decreased to within the limits of normal values—values supplied independently by the observations of Richmond and co workers (12) as well as McCrory and associates (10) Phosphate clearances were elevated at 4 and 6 months if the normal phosphate clearance value as used by Richmond and co workers is considered representative for that age group

The ratio of calcium to creatinine indicates a lowered urinary calcium excretion at 4 and 6 months of age

Avioli and associates (1) that patients with VDRR of the familial type cannot metabolize vitamin D properly into an active form. Such a defect could be present in the neonatal period but may be obscured by the treatment of the mother with high doses of vitamin D.

## SUMMARY

Survey of all available data of infants with familial hypophosphatemic vitamin D resistant rickets observed partially or throughout the first year of life indicates that hypophosphatemia begins in the neonatal period regardless of whether or not the mother had been treated with vitamin D in high doses. Growth failure in vitamin D resistant rickets cannot be prevented even when treatment with high doses of vitamin D is begun early.

## ACKNOWLEDGMENTS

Dr. Genevieve Stearns supplied the data for case 7 and Dr. Laurence Finberg for case 8.

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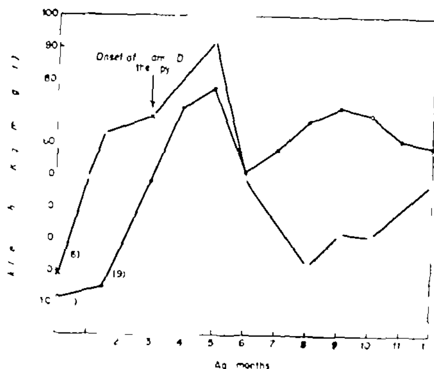



Fig 3 Alkaline phosphatase levels in two patients with VDRR (cases 6 and 9) whose treatment began at 3 months of age The level at birth in one patient (case 7) also can be seen

cently we studied one patient with VDRR who had normal height This girl was first seen when she was 11 1/ years old at which time she was 147 cm tall this placed her in the 50th percentile for her age She had VDRR and never had received high doses of vitamin D Slight bowing of her legs had been present since she began to walk

Observation of normal or nearly normal growth in an occasional VDRR patient (either treated or untreated) does not invalidate the fact that growth is retarded in the majority of affected children regardless of vitamin D therapy Increased intake of phosphate might affect growth as was pointed out by Harrison and his group (8) but this was not tried on any of the patients surveyed in this study It is important to note that growth retardation usually does not begin until the age of weight bearing when the affected individual begins to stand

All patients so far studied during the first year of life had hypophosphatemia that began in the newborn period The observation of hypophosphatemia indicated that the metabolic defect of VDRR is present at birth Why then are the results of neonatal roentgenographic studies normal and the level of alkaline phosphatase within normal limits? Without having results of such studies available in a patient with familial VDRR whose mother had not been treated no definite answer can be given However one may speculate that vitamin D therapy given to the mother prevents bone abnormalities in the fetus and therefore prevents elevation of alkaline phosphatase levels

Less conclusive in the documentation of the metabolic changes in the neonatal period are the urinary excretion studies The level of phosphate clearance does not seem to be elevated at birth but it is probably high by the age of 4 months and certainly is high at 6 months of age There is a trend that the urinary phosphate excretion in 24 hours is normal or low during the first 6 months of life this trend is similar to that seen in older children who had not been treated Furthermore hypocalciuria probably is present beginning at 1 month of age just as seen in untreated older children with VDRR However the observations need further confirmation before any conclusions can be drawn

None of the observations discussed in this paper contradicts the hypothesis advanced by

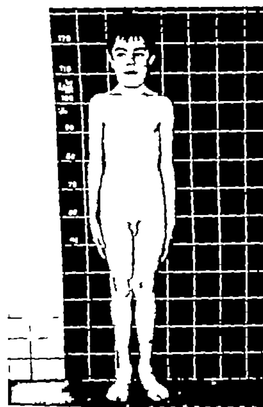


*Fig 1* Patient Pedro P

Constantia P now 19 years old is deaf and an inmate in the same institution as her brother Pedro She is short her height being below the tenth percentile (1 48 m) the weight is normal (50 4 kg) The *facial* features are coarse the nose is broad the jaw is square-shaped lips and tongue are thick the palate is high arched teeth show multiple caries Fundi and cornea are normal No hepatosplenomegaly bone deformities or stiffness of the joints are noticed Puberty development is normal Mental development is considerably impaired and at 19 years corresponds to an age of at most 5 years according to the Columbia test In the peripheral blood 22 % of the lymphocytes show a vacuolized cytoplasm without metachromatic granules

Urinary excretion of uronic acid is normal (1 7 mg per liter) Chromatographic fractionation of mucopolysaccharides also performed twice discloses the presence of heparitin sulphate and of both chondroitin sulphates A C and B

X ray alterations are less evident than in the brother Trabeculation of the bones appears normal The skull is brachycephalic with a small basal angle dense calvarium poorly aerated mastoid cells a normally shaped sella turcica and relatively exaggerated development of the maxillae The spinal column shows the typical aspect of Scheuermann's disease

## ENZYME STUDIES

Liver specimens were obtained through needle biopsy The enzyme studies were performed by Van Hoof & Hers (39) The results are presented in Table 1

## MORPHOLOGICAL STUDIES

The material was fixed for 90 min at 3 C in 4 2 % glutaraldehyde buffered at pH 7 4 according to Millonig After rinsing for 15 h at 3 C in Millonig's buffer 0 1 M containing 0 45 g glucose per 100 ml it was post fixed in 2 % osmium tetroxide (Millonig's glucosed buffer) for 30 min The fragments were then dehydrated with alcohol and embedded in Epon For light microscopy semi thin sections were stained with toluidine blue Ultra thin sections contrasted with lead according to Karnovsky's technique (13) and covered with a carbon film were examined with a Siemens Elmiskop I

### *Light microscopy*

Hepatocytes were found to contain numerous clear vacuoles of varying size (Fig 2) Similar inclusions but smaller and frequently more numerous were also observed in Kupffer cells This process was generalized in the boy whereas some hepatocytes were spared in the girl

### *Electron microscopy*

The different cell types of the liver display important lesions Hepatocytes are less affected in the girl than in the boy whereas in both cases there are severe alterations of Kupffer cells and

Table 1 *Results of enzyme studies*

| Enzym | Constancia U/g* | Constancia of normal values | Pedro U/g* | Pedro of normal values |
|---|---|---|---|---|
| Acid β galactosidase | 7 50 | 1 00 | 2 20 | 350 |
| N acetyl β glucosaminidase | 16 00 | 668 | 9 66 | 404 |
| β glucuronidase | 2 78 | 589 | 1 06 | 274 |
| Acid glucosidase | 2 76 | 393 | 3 44 | 490 |
| Acid β glucosidase | 0 19 | 177 | — | — |
| Acid galactosidase | 0 26 | 477 | — | — |
| Acid phosphatase | — | — | 1 17 | 63 |

Units per g of liver tissue

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# CLINICAL BIOCHEMICAL AND ULTRASTRUCTURAL STUDIES OF AN ATYPICAL FORM OF MUCOPOLYSACCHARIDOSIS

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During recent years much knowledge concerning mucopolysaccharidoses has accumulated through biochemical (2–4 7 12 15–17 19–21 28 30 33) enzymological (37–39) and electron microscopic (1 5 9 11 14 35 36 40) contributions Many studies have led to the separation of various types of chondrodystrophies corresponding to different clinical biological and radiologic pictures and characterized by an increased urinary excretion of one or more mucopolysaccharides (17 21)

However besides descriptions typical for each of these mucopolysaccharidoses there are some observations that cannot be as easily classified Sometimes the clinical picture is atypical or does not agree with the biochemical findings in the urines (19 24 25 29) In other cases clinical examination shows signs of both gargoylism and lipidosis and biochemical analysis of the tissues discloses a generalized accumulation of a ganglioside (12 15 22 27 31 32)

We have recently observed two children brother and sister presenting clinically a peculiar form of mucopolysaccharidosis Studies of liver tissue obtained by needle biopsies have shown an enzyme pattern and ultrastructural picture different from those previously described It seems of interest to report these two observations and to discuss briefly the significance of the biochemical and ultrastructural data

## CASE REPORTS

Pedro P (Fig 1) now 10 years old was seen for the first time when aged 9 He is an inmate in an institution for deafmute children deafness having appeared in early infancy The parents are healthy and not related and there is no evident family history of gargoylism His height and weight are normal (1 78 m and 27 kg) His head is enlarged Facial features are coarse however not showing the typical gargoyle aspect tongue and lips are thick the nasal bridge is flattened Hypertelorism low set ears and a slight inferior prognathism are noted There is a defective implantation of the teeth which show widespread caries Fundi and cornea are normal The thorax shows pigeonbreast deformity A slight dorsal kyphosis is noticed Cardiac examination is normal Abdomen is protruding however without hepatosplenomegaly or hernia There is a slight stiffness of the joints without true limitation of extension Mental development is considerably delayed and at 10 years corresponds to an age of at most 5 / years according to the Columbia test In the peripheral blood no abnormal granulations are observed in the polymorphonuclear leukocytes but 60 % of the lymphocytes show a vacuolized cytoplasm These vacuoles do not stain either with May Grunwald Giemsa or with toluidine blue

Urinary excretion of uronic acid (precipitation of mucopolysaccharides with cetyltrimethylammonium bromide and determination of the hexuronic acid content by the carbazole reaction (8)) is very high (22 mg per liter) Chromatographic fractionation of mucopolysaccharides performed twice according to a previously described technique (9) shows the presence of abnormally high quantities of heparitin sulphate as well as of both chondroitin sulphates A–C and B

X ray findings show a moderately delayed skeletal maturation (bone age 7 years chronological age 9 years) Trabeculation of the bones is slightly decreased tubular bones showing a laminated corticalis The skull is brachycephalic with a small basal angle a dense calvarium poorly aerated sinuses a normally shaped sella turcica and a relatively exaggerated development of the maxillae Vertebral bodies are biconvex from D6 to L2 the inferior surface of L2 being irregular The sacro sciatic angle is acute there is a slight bilateral coxa valga The ribs are spatulated The distal ends of the metacarpals are club shaped and the metatarsals are bulky

Fig. 4. C.P. Hepatocyte. Two inclusions similar to those described in Fig. In one of them myelin-like figures are seen (ml). m mitochondria, g glycogen, er ergastoplasm (Glut. OsO. Epon. Karnovsky.) 24 000

*Fig. 5.* C.P. Hepatocyte containing a large inclusion in which the spherules are very numerous and almost juxtaposed. n nucleus, m mitochondria, g $\alpha$-glycogen (Glut. OsO. Epon. Karnovsky.) 16 000

*Fig 2* P P Light microscopy H patocytes and Kupffer cells contain numerous clear vacuoles of varying size *H* hepatocyte *K* Kupffer cell *n* nucleus (Glut OsO Epon toluidine blue)

*Fig 3* P P H patocyte Two clear inclusions containing a delicate protein like pr cipitate numerous dense granules (*dg*) and spherules (*s*) with a clear center and an osmiophilic limit *m* mitochondria *g* OsO Epon Karnovsky) 22 200

tocytes (Fig 6) They were however smaller in size (0 3μ to 4μ) and their contents were somewhat different the clear matrix was abundant with its protein like precipitate uniformly dispersed some very osmophilic granules were scattered throughout but there were no spherules with clear center and dense limits The cytoplasm was stretched into thin trabeculae The usual cell organelles appear normal

Between the hepatocytes and the Kupffer cells the spaces of Disse do not reveal any obvious alterations (Fig 6)

*Atypical groups of cells* Groups of ballooned cells were observed with discontinuous plasmatic membranes making their limits poorly distinguishable An extracellular space similar to the space of Disse isolates these groups of cells from adjacent hepatocytes Their cytoplasm is homogenous and contains rather numerous particles of α glycogen Endoplasmic reticulum appears dislocated Mitochondria and Golgi apparatus are normal Frequently blood cells are seen in direct contact with the cytoplasm

This cell type is particularly distended with clear inclusions varying in sizes (0 3μ to 3 5μ) They are similar to those observed in Kupffer cells

## DISCUSSION

The clinical picture of these two patients fits rather well with that of mucopolysaccharidosis type III (21) significant mental retardation (especially in the girl) inconspicuous facial and bone deformities absence of corneal clouding of hepatosplenomegaly and stiffness of the joints presence of cytoplasmic vacuoles in the lymphocytes The fact that typical bone lesions are absent in the girl should be stressed Relevant to this finding is the report of Spranger *et al* (30) who mention in one case an obvious regression of some radiological abnormalities

The diagnosis of Sanfilippo's disease is invalidated by the qualitative analysis of urinary mucopolysaccharides which discloses in the probands on two different occasions the presence of both heparitin sulphate and chondroitin sulphate B Taking into account these data and also the fact that a brother and a sister from a family without known history of gargoylism are simultaneously affected the diagnosis of mucopolysaccharidosis type I should be suspected However the clinical manifestations are less severe than in classical Hurler's disease Finally it should be pointed out that quantitative determination of urinary mucopolysaccharide expressed in mg uronic acid disclose a normal excretion in the girl a fact which has also been reported by Maroteaux & Lamy (18) and by McKusick *et al* (20) in atypical cases termed pseudo-Hurler

The discrepancy which exists between clinical and radiological data and biochemical findings is such that our two patients cannot be classified into the actually known types of mucopolysaccharidoses Similar conclusions are reached by Pena *et al* (24) and by Clausen *et al* (6) who report patients showing the typical clinical picture of Hurler's disease and urinary excretion of chondroitin sulphate B exclusively The 2 observations of Sarrouy *et al* (29) and of Rampini & Maroteaux (25) should also be mentioned They concern cases of polydystrophic dwarfism with skeletal deformities different from those usually observed in mucopolysaccharidosis type III and possibly corresponding to a new phenotype of Hurler's syndrome

As regards enzyme studies of the liver our patients also present an unusual type of mucopolysaccharidosis Some of the lysosomic acid hydrolases show a definite higher than normal activity as frequently observed in gargoylism Moreover there exists a striking hyperactivity of acid β galactosidase whereas in all other cases studied by Van Hoof & Hers (39) the activity of this enzyme is either normal or strongly decreased Such a high activity of β galactosidase has also been found by Öckerman (23) in a generalized storage disorder resembling Hurler's syndrome in this case α mannosidase activity was decreased in the liver spleen and brain cortex and total




*Fig 6* C P Kupffer cell The cytoplasm is distended by inclusions *H* hepatocyte *D* space of Disse *S* sinusoid (Glut OsO Epon Karnovsky) 24 000

of groups of cells whose precise nature cannot be accurately defined

*Hepatocytes* Most of the nuclei appeared normal rarely they contained $\beta$ glycogen Mitochondria Golgi apparatus and endoplasmic reticulum—smooth and granular—were normal Cytoplasm contained $\alpha$ glycogen Some inclusions were observed whose contents show morphological characteristics of neutral lipids Dense bodies were inconstantly found in the girl and are absent in the boy The cytoplasm was filled with numerous inclusions varying in size from 0 2 to 7$\mu$ limited by a unit membrane (Figs 3 4 5) Their contents were heterogenous The main constituent is a clear amorphous matrix in which more osmiophilic structures may be distinguished

1 A rather faintly stainable substance uniformly dispersed as a fine protein like precipitate (Figs 3 4)
2 Small scanty membranous elements which form myelin like figures (Fig 4)
3 Dense small granules (0 02 $\mu$ to 0 2 $\mu$) (Figs 3 4 5)
4 Larger spherules (0 03 $\mu$ to 0 6 $\mu$) with a clear center and an osmiophilic limit, whose ultrastructure resembles that of neutral lipids (Figs 3 4 5)

The clear matrix generally abundant is however sometimes scanty in certain inclusions The spherules are then numerous and almost juxtaposed (Fig 5) The alterations of the hepatocytes are less conspicuous in the girl than in the boy inclusions are generally smaller (0 2$\mu$ to 6$\mu$) and only observed in some of the cells

*Kupffer cells* They were generally swollen and filled most of the lumen in the sinusoid The cytoplasm was filled with numerous inclusions similar to those described in the hepa

tocytes (Fig 6) They were however smaller in size (0 3μ to 4μ) and their contents were somewhat different the clear matrix was abundant with its protein like precipitate uniformly dispersed some very osmiophilic granules were scattered throughout but there were no spherules with clear center and dense limits The cytoplasm was stretched into thin trabeculae The usual cell organelles appear normal

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mannose was strikingly increased in the liver In the absence of α mannosidase and mannose determinations in our patients liver and of electron microscopic studies in Ockerman s case no definite conclusions can be drawn concerning the possible correlations between these observations

Finally the liver ultrastructure presents an appearance rather different from that previ ously described Many recent cytochemical (14 41) and electron microscopic (5 9 34 36 40) studies have described the morphology of the hepatic lesions in mucopolysaccharidoses In Hurler s Hunter s and Sanfilippo s syndromes hepatocytes and Kupffer cells are filled with numerous bodies containing mucopolysaccharides These inclusions present in all cells, are probably of lysosomic nature They reach diameters up to 10μ are limited by a unit membrane and contain an electon lucid matrix in which a finely granular protein like precipitate is scattered In some of them a few more osmiophilic spherules generally adjacent to the membrane are observed In Morquio s disease a similar aspect is observed in Kupffer cells while hepatocytes appear al most normal (34) The ultrastructural aspect is also quite different from that described by Durand *et al* (10) and also found by us[1] in cases of mucopolysaccharidosis by absence of α fucosidase (38) In this last disea e the liver cells are filled with numerous inclusions Some of them contain mainly a protein like reticulum while others display strongly osmiophilic spherules whose contents have a periodic aspect characteristic of complex lipids

In the present cases the storage process is not very intense The vacuoles are seen in the different cell types and their diameters do not exceed 7μ In the girl moreover many hepatocytes seem unaltered Also the inclusions differ with respect to their contents they are limited by a unit membrane and contain an electron lucid matrix in which a finely ar ranged protein like precipitate is scattered The hepatocytes contain in addition numerous dense granules and spherules varying in osmiophily probably of neutral lipid nature This last component, analogous to that observed in limited number in the typical forms, is sometimes so abundant that the clear matrix is al most non existent

The atypical cell groups are not mentioned in ultrastructural descriptions of normal liver (26) On the other hand a similar aspect has been described in Morquio s disease (34) It is possible that technical manipulations may induce membrane ruptures of overloaded cells and consequently a cytoplasmic flow in adja cent spaces

[1] Unpublished data

## SUMMARY

Two children brother and sister presenting a clinically peculiar form of mucopolysaccharid osis are reported There exists a discrepancy between clinical and radiological data and biochemical findings Enzyme study of the liver discloses a striking hyperactivity of the acid β galactosidase Hepatic ultrastructure differs from that of other previous descriptions of mu copolysaccharidoses demonstrating a complex storage of lipids and mucopolysaccharides within swollen lysosomes

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# THE IMMUNOGLOBULIN DEVELOPMENT DURING THE FIRST YEAR OF LIFE

*A Longitudinal Study*

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In a previous paper (11) the results were reported of immunoglobulin determinations in healthy Swedish children from the neonatal period up to the age of 5 years. The children investigated in this previous study were completely healthy and had only undergone a few mild infections. On studying the literature we found that previously published studies of immunoglobulin levels in children had yielded varying results (2, 6, 7, 15, 16) even though the different immunoglobulin concentrations were expressed in relation to a stated mean adult level so that the discrepancies concerning methods of determination and immunoglobulin standard could be largely disregarded.

The children studied in our previous investigation were selected in such a way that they could be regarded as representing a completely healthy population. It was considered that the mode of selection resulted in lower mean immunoglobulin levels than would have been obtained in a paediatric series including children who were healthy but whose immunoglobulin production had been stimulated by repeated, predominantly trivial infections. In this previous investigation, however, we were mainly interested in the lower limit values of different immunoglobulins in healthy children. As a complement to this investigation a report is given below of a longitudinal study of the immunoglobulin development during the first year of life. The aim of the present investigation was twofold: firstly to make a detailed study of the immunoglobulin development in the individual child, and secondly to obtain an idea of the importance of the state of health of a child, especially its history of infections, for its mean immunoglobulin concentrations during the first year of life.

## MATERIAL AND METHODS

Altogether 34 fully developed infants who had shown no signs of disease in the maternity clinic were selected for repeated immunoglobulin determinations during their first year of life. Samples were taken during the first 24 hours after delivery, on a few more occasions during their stay in the maternity clinic, at the age of 3 weeks and subsequently at the same ages as in the Baby Welfare Centre (BWC) series of the previous investigation, i.e. 6 weeks, 3 months, 4 1/2 months, 6 months, 9 months and 1 year. For only 1 child was the investigation discontinued, and this was at an early stage when the mother had requested that no further samples should be taken. The samples were taken at the infants' homes by a nurse, and on each occasion of sample taking a brief history was taken with especial regard to past infections of different kinds. Free medical care was offered for the infants when required, and this was utilized to a large extent. A fairly good idea was thus obtained of the history of infections in these infants. Of the 33 infants who were thus followed up until the age of 1 year, none showed signs of any systemic disease or other serious illness of any kind. A few of the infants had mild atopic eczema. As expected, several of the infants underwent slight infections, including infections of the upper respiratory tract, bronchitis and otitis.

In a further 7 infants repeated blood samples were taken during the first 3 weeks of life with the aim of studying the development of the IgM concentrations. Samples were taken at birth, 24, 48, 72, 96 and 120 hours after birth and subsequently at the ages of 1, 2 and 3 weeks.

39 Van Hoof F & Hers H G The abnormalities of lysosomal enzymes in mucopolysaccharidosis *Europ J Biochem 7* 34 1968
40 Wallace B J Kaplan D Adachi M Schneck L & Volk B W Mucopolysaccharidosis type III Morphologic and biochemical studies of two siblings with Sanfilippo syndrome *Arch Path 82* 462 1966
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*Key words* Mucopolysaccharidosis atypical enzyme pattern hepatic ultrastructure

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# THE IMMUNOGLOBULIN DEVELOPMENT DURING THE FIRST YEAR OF LIFE

*A Longitudinal Study*

TORSTEN BERG

*From the Department of Pediatrics and the Blood Center University Hospital Uppsala Sweden*

In a previous paper (11) the results were reported of immunoglobulin determinations in healthy Swedish children from the neonatal period up to the age of 5 years. The children investigated in this previous study were completely healthy and had only undergone a few mild infections. On studying the literature we found that previously published studies of immunoglobulin levels in children had yielded varying results (2 6 7 15 16) even though the different immunoglobulin concentrations were expressed in relation to a stated mean adult level so that the discrepancies concerning methods of determination and immunoglobulin standard could be largely disregarded.

The children studied in our previous investigation were selected in such a way that they could be regarded as representing a completely healthy population. It was considered that the mode of selection resulted in lower mean immunoglobulin levels than would have been obtained in a paediatric series including children who were healthy but whose immunoglobulin production had been stimulated by repeated predominantly trivial infections. In this previous investigation however we were mainly interested in the lower limit values of different immunoglobulins in healthy children. As a complement to this investigation a report is given below of a longitudinal study of the immunoglobulin development during the first year of life. The aim of the present investigation was twofold firstly to make a detailed study of the immunoglobulin development in the individual child and secondly to obtain an idea of the importance of the state of health of a child especially its history of infections for its mean immunoglobulin concentrations during the first year of life.

## MATERIAL AND METHODS

Altogether 34 fully developed infants who had shown no signs of disease in the maternity clinic were selected for repeated immunoglobulin determinations during their first year of life. Samples were taken during the first 24 hours after delivery on a few more occasions during their stay in the maternity clinic at the age of 3 weeks and subsequently at the same ages as in the Baby Welfare Centre (BWC) series of the previous investigation i.e. 6 weeks 3 months 4 1/2 months 6 months 9 months and 1 year. For only 1 child was the investigation discontinued and this was at an early stage when the mother had requested that no further samples should be taken. The samples were taken at the infants homes by a nurse and on each occasion of sample taking a brief history was taken with especial regard to past infections of different kinds. Free medical care was offered for the infants when required and this was utilized to a large extent. A fairly good idea was thus obtained of the history of infections in these infants. Of the 33 infants who were thus followed up until the age of 1 year none showed signs of any systemic disease or other serious illness of any kind. A few of the infants had mild atopic eczema. As expected several of the infants contracted slight infections including infections of the upper respiratory tract, bronchitis and otitis.

In a further 7 infants repeated blood samples were taken during the first 3 weeks of life with the aim of studying the development of the IgM concentrations. Samples were taken at birth, 4 48 72 96 and 120 hours after birth and subsequently at the ages of 1 2 and 3 weeks.

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## RESULTS

*IgG* As can be seen in Fig 1 from the age of $4^1/_2$ months onwards the infants who had undergone recurrent infections of different kinds exhibited throughout the highest mean IgG levels The difference from the mean value for the entire s ries was however very small and on no occasion exceeded 75 mg/100 ml (maximally 16 above the mean at the age of 6 months) The IgG curve for the infants in the previous BWC series is in fairly good agreement with the mean IgG curve for the present series as a whole while those infants in the present series who had been free or practically free from infections from the age of 3 months showed throughout the lowest IgG levels although the differences were small On comparing the mean IgG level between the ages of 4 / months and 1 year in the 5 infants with recurrent infections with that in the 10 infants who were free or practically free from infections a difference was found which was however not significant ($p \approx 0.08$)

In Fig. 2 the group of 5 infants who had the highest and the group of 5 with the lowest IgG

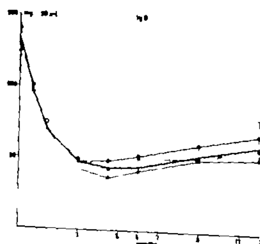


*Fig 1* The mean IgG development ±1 *s.D.* in the whole series (———) This is compared with the IgG development in 5 infants with recurrent infections (○——○) 10 infants who wer practi ally free from infections (△—△) and a previously studied series of healthy children from a Baby Welfare Centre (●– – –●)

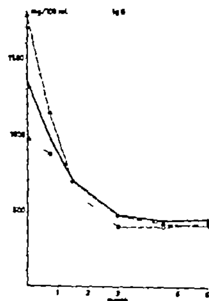


*Fig 2* The IgG development in the 5 infants who had the highest IgG concentrations at birth (●– – –●) compared with that in the 5 infants with the lowest IgG concentrations at birth (○– – –○) and also with th mean IgG development in the whole series

concentrations in capillary blood at birth are compared In 3 infants umbilical cord sera were used for the IgG determination at birth As there seems to be a systematic difference between IgG concentrations in umbilical cord blood and in capillary blood at birth (4) these infants were not included in this comparison The mean difference was initially considerable almost 800 mg/100 ml (58 of the mean value at birth in the whole series) As shown in the figure the difference between these two groups diminished rapidly and when the infants were only 6 weeks old the difference was hardly 60 mg/100 ml (8% of the mean value for the age in whole series) Subsequently the IgG curves for the two groups become increasingly similar The decrease in IgG concentration from birth to the age of 6 weeks was significantly more rapid in the infants with highest initial values than in those with the lowest values at birth ($p < 0.001$) Approximately the same level of significance was obtained when comparing extreme groups comprising 5 6 7 and 8 infants

Table 1 *Immunoglobulin concentrations in mg/100 ml (average levels* S D *and range)*

| Age | 0 days | | | 3 weeks | | | 6 weeks | | | 3 months | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IgG | IgA | IgM | IgG | IgA | IgM | IgG | IgA | IgM | IgG | IgA | IgM |
| *All 33 infants* | | | | | | | | | | | | |
| Mean | 1359 | | 9 8 | 980 | 4 6 | 41 9 | 697 | 7 1 | 38 9 | 474 | 11 0 | 45 8 |
| S D | 268 | | 5 1 | 155 | 4 5 | 23 6 | 112 | 5 0 | 28 6 | 232 | 6 5 | 19 4 |
| Range | 823– | | 3 1– | 585– | 1 3– | 13 2– | 510– | 1 7– | 10 7– | 229– | 2 0– | 14 9– |
| | –2148 | | –22 2 | –1286 | –26 0 | –92 4 | –1020 | –22 7 | –146 2 | –1423 | –28 2 | –113 7 |
| *10 infants free from infection* | | | | | | | | | | | | |
| Mean | 1419 | | 8 8 | 967 | 3 5 | 33 4 | 702 | 7 4 | 31 1 | 432 | 12 2 | 37 1 |
| Range | 1144– | | 3 6– | 585– | 1 8– | 13 2– | 510– | 1 7– | 10 7– | 278– | 2 7– | 14 9– |
| | –2148 | | –15 1 | –1133 | –6 2 | –67 0 | –846 | –12 7 | –52 1 | –672 | –28 2 | –68 1 |
| *5 infants with recurrent infect* | | | | | | | | | | | | |
| Mean | 1280 | | 10 5 | 1002 | 8 8 | 46 8 | 751 | 11 1 | 69 1 | 487 | 14 8 | 65 6 |
| Range | 1031– | | 3 1– | 795– | 1 4 | 19 6– | 665– | 4 3– | 29 4– | 229– | 10 4– | 44 7 |
| | –1661 | | –22 2 | –1286 | –26 0 | –92 4 | –835 | –16 3 | –146 2 | –708 | –23 3 | –113 7 |

| Age | 4½ months | | | 6 months | | | 9 months | | | 1 year | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IgG | IgA | IgM | IgG | IgA | IgM | IgG | IgA | IgM | IgG | IgA | IgM |
| *All 33 infants* | | | | | | | | | | | | |
| Mean | 436 | 14 7 | 51 5 | 453 | 15 8 | 53 6 | 542 | 21 0 | 61 0 | 616 | 26 6 | 70 4 |
| S D | 238 | 9 4 | 24 3 | 203 | 8 8 | 20 1 | 201 | 10 0 | 21 0 | 198 | 16 1 | 26 7 |
| Range | 231– | 2 9– | 18 6– | 213– | 4 4– | 18 6– | 270– | 5 3– | 23 8– | 328– | 7 2– | 33 9– |
| | –1235 | –37 7 | –140 4 | –1093 | –38 5 | –110 4 | –890 | –47 8 | –115 0 | –1010 | –65 7 | –176 6 |
| *10 infants free from infection* | | | | | | | | | | | | |
| Mean | 367 | 15 0 | 36 8 | 420 | 14 2 | 43 8 | 518 | 20 0 | 57 8 | 525 | 17 6 | 56 9 |
| Range | 231– | 2 9– | 18 6– | 220– | 4 4– | 18 6– | 270– | 5 3– | 23 8– | 328– | 7 2– | 34 1 |
| | –578 | –37 7 | –56 7 | –645 | –35 8 | –69 6 | –811 | –34 8 | –87 0 | –790 | –41 1 | –92 7 |
| *5 infants with recurrent infect* | | | | | | | | | | | | |
| Mean | 490 | 15 4 | 78 7 | 525 | 24 1 | 78 7 | 617 | 25 9 | 75 5 | 691 | 44 6 | 109 2 |
| Range | 373– | 8 7– | 52 1– | 272– | 15 9– | 42 6– | 423– | 16 0– | 58 8– | 458– | 19 1– | 87 [illegible] |
| | –740 | –24 0 | –140 4 | –708 | –38 5 | –110 4 | –890 | –34 2 | –87 6 | –958 | –65 7 | –176 6 |

Finally in 2 infants who during the first 5 days of life had been tended under conditions as free from bacteria as possible blood samples were taken in the same way as in the 7 infants mentioned above but only up to the age of 2 weeks These infants who were delivered by caesarian section were tended after the delivery by staff wearing sterilized clothes and were subsequently nursed in an incubator with sterile bedding and fed on sterilized breast milk Repeated bacterial cultures from the faeces nose and throat skin and umbilicus were taken at birth and during the first 5 days In one of the infants all of these cultures were negative during the first 2 days but from the third day onwards staphylococcus albus was observed repeatedly in cultures from different localizations and from the age of 5 days coliform bacteria were also seen in the faeces The other child showed negative cultures only during the first day after which staphylococcus albus was observed in the cultures from all localizations

With a few exceptions the blood samples taken consisted of capillary blood from the heel or finger tip and after coagulation and centrifugation the sera were stored at –20 C until analysed Quantitative determinations of the different immunoglobulins were made by single radial immunodiffusion in agar gel using a modification (10) of the method of Mancini *et al* (12) In some infants a few samples had to be missed since at the intended time of sample taking the family was away on vacation In 2 infants analysis of one isolated blood sample gave unreasonably high IgA values (>200 mg/100 ml) The IgA development in these infants did not differ in other respects from the average *they showed no signs of any* disease at the time of sample taking and these results were not included in the statistical calculations

## RESULTS

*IgG* As can be seen in Fig 1 from the age of 4½ months onwards the infants who had undergone recurrent infections of different kinds exhibited throughout the highest mean IgG levels The difference from the mean value for the entire s ries was however very small and on no occasion exceeded 75 mg/100 ml (maximally 16 above the mean at the age of 6 months) The IgG curve for the infants in the previous BWC series is in fairly good agree ment with the mean IgG curve for the present series as a whole while those infants in the present series who had been free or practically free from infections from the age of 3 months showed throughout the lowest IgG levels al though the differences were small On compar ing the mean IgG level between the ages of 4½ months and 1 year in the 5 infants with recurrent infections with that in the 10 infants who were free or practically free from infec tions a difference was found which was how ever not significant ($p \approx 0.08$)

In Fig 2 the group of 5 infants who had the highest and the group of 5 with the lowest IgG

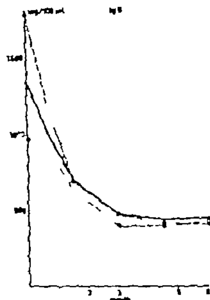


*Fig 2* The IgG development in the 5 infants who had the highest IgG concentrations at birth (● – – – ●) compared with that in the 5 infants with the lowest IgG concentrations at birth (○ – – – ○) and also with the mean IgG development in the whole series.

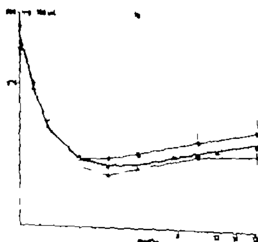


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Table I *Immunoglobulin concentrations in mg/100 ml (average levels ± S.D. and range)*

| Age | 0 days | | | 3 weeks | | | 6 weeks | | | 3 months | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IgG | IgA | IgM | IgG | IgA | IgM | IgG | IgA | IgM | IgG | IgA | IgM |
| *All 33 infants* | | | | | | | | | | | | |
| Mean | 1359 | | 9 8 | 980 | 4 6 | 41 9 | 697 | 7 1 | 38 9 | 474 | 11 0 | 45 8 |
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| *10 infants free from infection* | | | | | | | | | | | | |
| Mean | 1419 | | 8 8 | 967 | 3 5 | 33 4 | 702 | 7 4 | 31 1 | 432 | 12 2 | 37 1 |
| Range | 1144– −2148 | | 3 6– −15 1 | 585– −1133 | 1 8– −6 2 | 13 2– −67 0 | 510– −846 | 1 7– −12 7 | 10 7– −57 1 | 278– −672 | 2 7– −28 2 | 14 9– −68 1 |
| *5 infants with recurrent infect* | | | | | | | | | | | | |
| Mean | 1280 | | 10 5 | 1002 | 8 8 | 46 8 | 751 | 11 1 | 69 1 | 487 | 14 8 | 66 6 |
| Range | 1031– −1661 | | 3 1– −22 2 | 795– −1286 | 1 4 −26 0 | 19 6– −92 4 | 665– −835 | 4 3– −16 3 | 29 4– −146 2 | 229– −708 | 10 4– −23 3 | 44 7 −113 7 |

| Age | 4½ months | | | 6 months | | | 9 months | | | 1 year | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IgG | IgA | IgM | IgG | IgA | IgM | IgG | IgA | IgM | IgG | IgA | IgM |
| *All 33 infants* | | | | | | | | | | | | |
| Mean | 436 | 14 7 | 51 5 | 453 | 15 8 | 53 6 | 542 | 21 0 | 61 0 | 616 | 26 6 | [illegible]0 4 |
| ± D | 238 | 9 4 | 24 3 | 203 | 8 8 | 20 1 | 201 | 10 0 | 21 0 | 198 | 16 1 | 26 7 |
| Range | 231– −1235 | 2 9– −37 7 | 18 6– −140 4 | 213– −1093 | 4 4– −38 5 | 18 6– −110 4 | 270– −890 | 5 3– −47 8 | 23 8– −115 0 | 328 −1010 | 7 2– −65 7 | 33 9 −136 6 |
| *10 infants free from infection* | | | | | | | | | | | | |
| Mean | 367 | 15 0 | 36 8 | 420 | 14 2 | 43 8 | 518 | 20 0 | 57 8 | 525 | 17 6 | 56 9 |
| Range | 231– −578 | 2 9– −37 7 | 18 6– −56 7 | 220– −645 | 4 4– −35 8 | 18 6– −69 6 | 270– −811 | 5 3– −34 8 | 23 8– −87 0 | 328– −790 | 7 [illegible]– −41 1 | 34 1 −92 7 |
| *5 infants with recurrent infect* | | | | | | | | | | | | |
| Mean | 490 | 15 4 | 78 7 | 525 | 24 1 | 78 7 | 617 | 25 9 | 75 5 | 691 | 44 6 | 109 2 |
| Range | 373– −740 | 8 7– −24 0 | 52 1– −140 4 | 272– −708 | 15 9– −38 5 | 4[illegible] 6– −110 4 | 423– −890 | 16 0– −34 2 | 58 8– −87 6 | 458– −958 | 19 1– −65 7 | 87 [illegible] −126 6 |

Finally in 2 infants who during the first 5 days of life had been tended under conditions as free from bacteria as possible blood samples were taken in the same way as in the 7 infants mentioned above but only up to the age of 2 weeks These infants who were delivered by cesarian section were tended after the delivery by staff wearing sterilized clothes and were subsequently nursed in an incubator with sterile bedding and fed on sterilized breast milk Repeated bacterial cultures from the faeces nose and throat skin and umbilicus were taken at birth and during the first 5 days In one of the infants all of these cultures were negative during the first 2 days but from the third day onwards staphylococcus albus was observed repeatedly in cultures from different localizations and from the age of 5 days coliform bacteria were also seen in the faeces The other child showed negative cultures only during the first day after which staphylococcus albus was observed in the cultures from all localizations

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who were delivered normally and nursed in the usual way is compared with the corresponding curves for 2 infants delivered by cesarian section and nursed for the following 5 days under conditions as free from bacteria as could be produced with the procedure described. As can be seen in the figure, the IgM curve for one of these infants is fairly similar to the mean curve for infants tended in the usual way. The curve for the other infant is considerably flatter, but the IgM level rises, however, from the age of 2 days and it can hardly be said that there is any pronounced deviation in this infant from the normal IgM development.

*IgA.* As shown in Fig. 6, the infants with recurrent infections of different kinds had higher IgA concentrations throughout than the mean for the whole series, and differed significantly ($p < 0.01$) from the infection free infants with regard to the mean concentration of IgA from the age of 3 weeks to 1 year. The children in the BWC series, like the infection free infants of the present series, showed lower values during the second six months of life than the mean for the whole of the present series. In Fig. 7 the individual IgA curves for the infants with recurrent infections are compared with the mean IgA curve for the children in the previously reported BWC series. The tendency is the same as for IgM, although the develop-

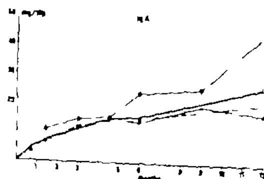


*Fig. 6.* The mean IgA development ±1 S.D. in the whole series (———). This is compared with the IgA development in 5 infants with recurrent infections (O——O), 10 infants who were practically free from infections (Δ——Δ) and a previously studied series of healthy children from a Baby Welfare Centre (● – – – ).

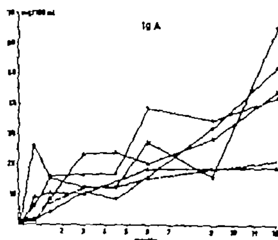




Fig. 7. The IgA development in 5 infants with recurrent infections compared with the mean IgA development in a previously studied series of children from a Baby Welfare Centre.

ment towards higher IgA concentrations seems to take place more gradually and no extremely high IgA values are noted during the first months of life.

*IgD.* At the age of 1 year this immunoglobulin was detected in only 3 of the 33 *infants*, which is in good agreement with the findings in previous investigations (11). In 2 of these infants IgD was observed at the age of 9 months and in 1 of these 2 it was also noted at 6 months.

## DISCUSSION

Previous investigations of immunoglobulin levels in children of different ages have given rather varying results. Stiehm & Fudenberg (15) thus found that adult levels of IgG and IgM were not reached until the age of 16 years while IgA slowly increased throughout childhood and at the beginning of the adult period.

Fulginiti and coworkers (7) reported that 1 year old children had attained, on the average, 75 % of the adult IgG level, 53 % of the adult IgM level and 20 % of the adult IgA level. The corresponding figures obtained by Allansmith et al. (2) were 73 %, 93 % and 24 %. In a few other investigations it is stated that the children had attained the adult IgM values before the age of 1 year (6, 16).

In the investigation carried out previously

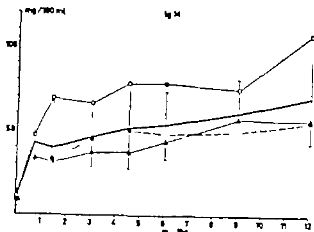


*Fig 3* The mean IgM development ±1 S.D. in the whole series (————) This is compared with the IgM development in 5 infants with recurrent infections (O——O) 10 infants who were practically free from infections (△——△) and a previously studied series of healthy children from a Baby Welfare Centre (● – – – ●)

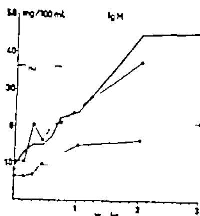


*Fig 5* The mean IgM development (————) in 7 infants during the first 3 weeks of life (O – – – O = the infant with the slowest IgM development) compared with the IgM development in 2 infants who during their first 5 days were nursed under conditions as free from bacteria as possible (●——●)

*IgM* The girls had at the ages of 9 months and 1 year somewhat higher IgM levels than the boys the difference however was not statistically significant Before the age of 9 months there was no systematic difference between the sexes As can be seen in Fig 3 the IgM levels in the infants in the present series were higher during the second six months of life than the mean values for the BWC series during this period The infants with recurrent infections had on the average, high IgM concentrations at a very early age, and the mean value in this group of infants lay consistently except on one occasion above the mean value +1 S.D. for the entire series The IgM levels in those infants who were practically free from infection lay throughout below the mean IgM levels for the whole series and at the age of 1 year these infants like the children in the BWC series showed an IgM level which was only about half of that in the infants with recurrent infections The mean concentrations of IgM from the ages of 3 weeks to 1 year were significantly higher in the 5 infants with recurrent infections than in the 10 infection free infants ($p<0.001$) As can be seen in Fig 4 which gives the separate IgM curves for the 5 infection sensitive infants these infants attained very high IgM concentrations at a very young age thus at the age of 6 weeks one of them showed a concentration of 146 mg/100 ml a value which lies in the upper part of the normal range for adults (10) From the age of 6 weeks only 2 of the 29 separate IgM values for the infants with recurrent infections lay below the mean IgM curve for the BWC series while 26 of the values were mostly well above this level

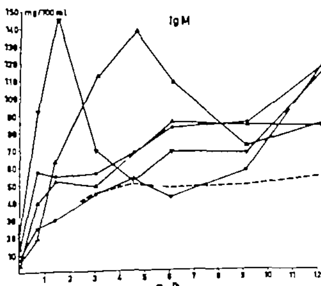


*Fig 4* The IgM development in 5 infants with recurrent infections compared with the mean IgM development in a previously studied series of healthy children from a Baby Welfare Centre

In Fig 5 the mean IgM curve for 7 infants

who were delivered normally and nursed in the usual way is compared with the corresponding curves for 2 infants delivered by cesarian section and nursed for the following 5 days under conditions as free from bacteria as could be produced with the procedure described As can be seen in the figure the IgM curve for one of these infants is fairly similar to the mean curve for infants tended in the usual way The curve for the other infant is considerably flatter but the IgM level rises however from the age of 2 days and it can hardly be said that there is any pronounced deviation in this infant from the normal IgM development

*IgA* As shown in Fig 6 the infants with recurrent infections of different kinds had higher IgA concentrations throughout than the mean for the whole series and differed significantly ($p < 0.01$) from the infection free infants with regard to the mean concentration of IgA from the age of 3 weeks to 1 year The children in the BWC series like the infection free infants of the present series showed lower values during the second six months of life than the mean for the whole of the present series In Fig 7 the individual IgA curves for the infants with recurrent infections are compared with the mean IgA curve for the children in the previously reported BWC series The tendency is the same as for IgM although the development towards higher IgA concentrations seems to take place more gradually and no extremely high IgA values are noted during the first months of life

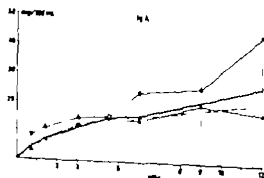



*Fig 6* The mean IgA development ±1 s.d. in the whole series (———) This is compared with the IgA development in 5 infants with recurrent infections (O——O) 10 infants who were practically free from infections (△——△) and a previously studied series of healthy children from a Baby Welfare Centre (● – – – )

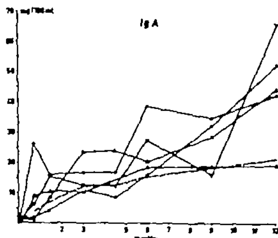



*Fig 7* The IgA development in 5 infants with recurrent infections compared with the mean IgA development in a previously studied series of children from a Baby Welfare Centre

*IgD* At the age of 1 year this immunoglobulin was detected in only 3 of the 33 infants which is in good agreement with the findings in previous investigations (11) In 2 of these infants IgD was observed at the age of 9 months and in 1 of these 2 it was also noted at 6 months

## DISCUSSION

Previous investigations of immunoglobulin levels in children of different ages have given rather varying results Stiehm & Fudenberg (15) thus found that adult levels of IgG and IgM were not reached until the age of 16 years while IgA slowly increased throughout childhood and at the beginning of the adult period

Fulginiti and coworkers (7) reported that 1 year old children had attained on the average 73 of the adult IgG level 53 of the adult IgM level and 20 of the adult IgA level The corresponding figures obtained by Allansmith *et al* (2) were 73% 93 and 24 In a few other investigations it is stated that the children had attained the adult IgM values before the age of 1 year (6 16)

In the investigation carried out previously

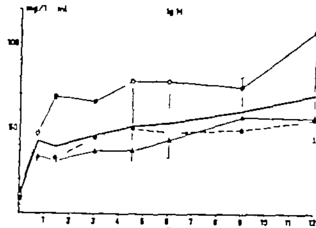



*Fig 3* The mean IgM development ±1 S D in the whole series (———) This is compared with the IgM development in 5 infants with recurrent infections (O——O) 10 infants who were practically free from infections (△——△) and a previously studied series of healthy children from a Baby Welfare Centre (● - - - ●)

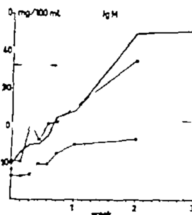



*Fig 5* The mean IgM development (———) in 7 infants during the first 3 weeks of life (O - - - O = the infant with the slowest IgM development) compared with the IgM development in 2 infants who during their first 5 days were nursed under conditions as free from bacteria as possible (●——●)

*IgM* The girls had at the ages of 9 months and 1 year somewhat higher IgM levels than the boys the difference however was not statistically significant Before the age of 9 months there was no systematic difference between the sexes As can be seen in Fig 3 the IgM levels in the infants in the present series were higher during the second six months of life than the mean values for the BWC series during this period The infants with recurrent infections had on the average high IgM concentrations at a very early age and the mean value in this group of infants lay consistently except on one occasion above the mean value +1 S D for the entire series The IgM levels in those infants who were practically free from infection, lay throughout below the mean IgM levels for the whole series and at the age of 1 year these infants, like the children in the BWC series showed an IgM level which was only about half of that in the infants with recurrent infections The mean concentrations of IgM from the ages of 3 weeks to 1 year were significantly higher in the 5 infants with recurrent infections than in the 10 infection free infants ($p<0.001$) As can be seen in Fig 4 which gives the separate IgM curves for the 5 infection sensitive infants these infants attained very high IgM concentrations at a very young age thus at the age of 6 weeks one of them showed a concentration of 146 mg/100 ml a value which lies in the upper part of the normal range for adults (10) From the age of 6 weeks only 2 of the 29 separate IgM values for the infants with recurrent infections lay below the mean IgM curve for the BWC series while 26 of the values were mostly well above this level

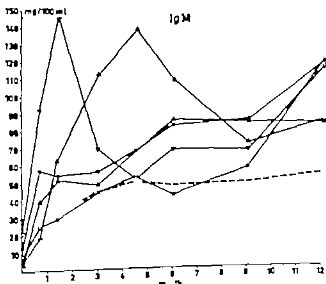



*Fig 4* The IgM development in 5 infants with recurrent infections compared with the mean IgM development in a previously studied series of healthy children from a Baby Welfare Centre

In Fig 5 the mean IgM curve for 7 infants

mg/100 ml from birth to the age of 3 weeks but then up to the age of 4 1/ months it lay at a practically unchanged level to decrease somewhat towards the end of the first year From these findings the conclusion may be drawn that in certain individuals a noteworthy IgG synthesis can take place relatively early in life

At a very early age a child has a capacity for a rapidly initiating and pronounced produc tion of IgM (Fig 4) which has been found previously in several investigations (3 14) In a number of children the IgM concentrations increase even during the first day of life while in others these concentrations remain relatively unchanged for one or a few days and then in crease The curve for the mean IgM values rises steeply up to the age of 2 weeks and then becomes flatter (Fig 5) or even falls slightly (2)

Of interest in this study were the large varia tions in the IgM concentrations which were found especially in the infants with recurrent infections In an investigation of healthy adults (1) who were studied once a week for 25 weeks a noteworthy stability of all immuno globulin concentrations was found on the other hand in the individual subject these concen trations were not affected noteworthily by up-per respiratory infections allergic symptoms smallpox vaccination or other factors studied

It seems possible that the rapid IgM increase during the first two weeks of life is partly related to the development of the normal in testinal flora It seemed therefore of great in terest to study the IgM development in infants fostered under sterile conditions In practice however this is very difficult to carry out satis factorily The two infants who were delivered by cesarian section and subsequently tended under conditions as free from bacteria as pos sible for 5 days both showed an increase in their IgM levels during this period In an in vestigation of the immunoglobulin levels in in fants with low birth weights (3) it was found that in the infants with the shortest gestational periods the IgM levels during the first weeks of life rose considerably more slowly than in the other infants It was considered that this could either be due to the fact that owing to the special care they had received they were bet ter protected from infections than other in fants or to a lower capacity for IgM synthesis in these immature infants The conclusion that can be drawn from the studies described here with sterile nursing of mature infants is that since the IgM development was not markedly delayed in these infants despite the extreme measures taken the slow IgM development in the premature infants could hardly be related to the care they had received but rather to their immaturity

In 10 infants i e just under 1/3 of the series investigated IgA was detected in small quan tities (1–3 mg/100 ml) during the first day of life This is in good agreement with the results of Stiehm & Fudenberg (15) In 7 of the infants IgA was detected on repeated sampling during the first week of life while in 3 infants IgA was only found during the first day possibly due to a small materno-foetal transfusion at the time of delivery when as is known IgA does not normally pass through the placenta (9) In all infants IgA was detected at the age of 3 weeks

## SUMMARY

In 33 infants repeated blood samples were taken during the first year of life for deter mination of the different immunoglobulins The infants were divided into groups according to their histories of infection These groups were compared with each other and as also was the series as a whole with a series of children investigated previously from a Baby Welfare Centre (BWC) A distinct relationship was found between the infants histories of in fection and their immunoglobulin develop-ment The history of infection appeared to be reflected predominantly in the serum concen trations of IgA and IgM and to a lesser extent in their IgG levels The initial IgG concentra tion appeared to be of little importance for the IgG development after the first weeks of life

on healthy Swedish children at a Baby Welfare Centre (11) a relatively slow immunoglobulin development was found during the first year of life At the age of 1 year IgG in these children was only about 45 of the mean adult level and the corresponding figures for IgM and IgA were 64 and 13 ° respectively These values agree fairly well with the results reported recently by Immonen (8)

In view of the fact that the infants of the present series were selected at the maternity clinic and that practically all of them could be followed up for the entire observation period, this can be regarded as *a purely non selected* series which should be fairly representative of Swedish children of this age group in an urban environment The group of 10 infection free infants in the present series should have corresponded most closely to the previously reported BWC series Five of the children had been particularly subjected to different infections and a further few children had such a history of infections that they would not have been included in the BWC series

As can be seen in the figures and table, there were more or less pronounced differences (*a*) between the BWC series and the present series and (*b*) between the different groups in the present series Those infants who had undergone repeated infections of different kinds exhibited throughout the highest concentrations of the respective immunoglobulins and at the age of 1 year they had reached 52 of the mean adult value for IgG 123 of the adult value for IgM and 28 of the adult value for IgA The corresponding figures for the infants who had been free or practically free from infections were 38 64 and 11 respectively values which on the whole agreed with or lay slightly below those obtained in the BWC series It thus seems as if the history of infections is of considerable importance for the rate at which the immunoglobulin development takes place during the first year of life In the series of infants reported here the levels of IgG appeared to reflect the history of infections to a smaller degree than those of IgA and IgM

Buckley *et al* (5) who studied sera from 600 children with recurrent infections compared the concentrations of the different immunoglobulins with those obtained earlier in a normal series and found deviations from the normal in a strikingly large number of the children, viz 44 • Of these children, 76 • had decreased concentrations of one or more immunoglobulins and 24 • increased concentrations

It can be difficult to decide whether an immunoglobulin concentration deviates pathologically from the normal if the deviation is not very pronounced *Completely healthy* children can apparently have strikingly low immunoglobulin concentrations (11) On the other hand the conclusion can be drawn from the series of children reported here that children who have undergone recurrent infections of different kinds but who are otherwise healthy can sometimes have remarkably high levels of especially IgM and IgA which need not necessarily then be regarded as pathological in themselves

With regard to development in the individual child it is found that the initial IgG concentration is of very little importance for the IgG development after the first weeks of life even very large differences in IgG levels between newborn infants seem to be largely evened out after a few weeks Obviously this may be due to a more rapid catabolism of maternal IgG in those children who have high concentrations at birth but another conceivable explanation is that their own synthesis of this immunoglobulin takes place more rapidly in children who initially have a low level of maternal IgG It is known that already in the foetus there is some capacity for IgG synthesis even though of quantitatively minor importance (13) In 2 of the infants of the present series unusual IgG curves were seen In one of them the IgG concentrations never decreased noteworthily and at the age of 3 months this child had an IgG value exceeding that at birth In the second child the serum concentration certainly decreased from about 1570–1020

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# A PATIENT WITH HEREDITARY GALACTOSEMIA STUDIED WITH A SCREENING METHOD FOR GALACTOSE IN URINE

A DAHLQVIST R JAGENBURG and A MARK

*From the Research Department of the Hospital University of Lund Lund Department of Paediatrics and Department of Clinical Chemistry University of Gothenburg Gothenburg Sweden*

The diagnosis of hereditary galactosemia[1] is often overlooked which may have serious consequences for the patient. Considerable effort is at present being made to find a suitable screening method for the detection of galactosemia in newborn infants (1 15 30 33). Recently we had a patient in which the diagnosis was stated 9 days after birth at which time the patient was in a very bad condition. Urine and blood were analyzed for galactose and amino acids with samples obtained immediately after the introduction of a galactose free diet. Quantitative analysis of galactose in blood and urine was performed and the urine was also analyzed with a recently developed galactose specific test paper. Analysis for galactitol and galactose 1 phosphate was performed. The diagnosis of hereditary galactosemia was confirmed by assay of galactose 1 phosphate uridyl transferase (E C 2 7 7 12) activity in blood samples both from the patient and from his parents

## CASE REPORT

The patient is a boy born on December 12th 1966. He is the first child of healthy parents without known consanguinity. Delivery was normal and the birth weight 3780 g. From the third day of life he suffered from an increasing degree of icterus and at the same time became indolent and difficult to feed. No blood group incompatibility could be demonstrated. At 6 days of age he had a serum bilirubin value of 29 5 mg/100 ml and his weight was 15 % below the birth weight. He was admitted to the University Hospital of Gothenburg on the 7th day of life. He had an increased muscular tonus and later got convulsions with opisthotonus. Lumbar puncture was performed and the liquor was found to have a high protein and cell content (208–232 mg % protein and 21 110 white blood cells/mm mainly mononuclear). Since meningitis could be suspected antibiotics were given. Between the 7th and 9th day of life exchange blood transfusion was performed three times but the serum bilirubin concentration still remained high. On the 8th day of life the boy began vomiting liver enlargement was noted and the cerebral symptoms became accentuated with lethargy and failure to eat

A positive test for reducing sugars in urine had been obtained already on the 6th day after birth. A repeated test was performed on the 7th day and also this was positive while at the same time Clinistix® was negative. Hereditary galactosemia was then suspected and special tests for this disease were performed on the 8th day of life (see below) which confirmed the diagnosis. A routine blood test for phenylalanine (screening with the Guthrie test for PKU) taken on the 5th day of life was reported positive 4 days later with a phenylalanine concentration of 8 mg/100 ml

Milk feeding was interrupted on the 9th day and replaced with intravenous invertose administration. From the 10th day of life a galactose free diet, Sobee® was introduced on the 11th day substituted by Galactomin®. With this treatment the patient improved dramatically

*Laboratory findings*

On admittance the hemoglobin concentration was 20 0 g/100 ml. GOT (glutamic-oxaloacetic acid transaminase) was 299 Karmen-Ordell units and GPT

[1] Hereditary galactose intolerance has been proposed as a more logical name for this disease (6). There exist however several earlier names for the disease and the nomenclature becomes still more complicated by the recent discovery of an additional form of galactosemia (galactose intolerance) due to galactokinase (E C 2 7 1 6) deficiency (17). We will therefore in this article use the name hereditary galactosemia which is at present the most generally accepted name

The infants who had the highest IgG levels at birth showed a significantly more rapid decrease in their IgG concentrations during the first 6 weeks of life than those with the lowest initial IgG levels This finding may possibly indicate a later start of their own synthesis of IgG in infants who have a high serum concentration of maternal IgG at birth The infants exhibited a very early capacity for IgM synthesis and the mean IgM curve rose steeply during the first two weeks of life subsequently becoming flatter The infants with recurrent infections of different kinds showed on the average twice as high IgM concentrations at the age of 1 year as the children in the previously reported BWC series

The IgA development took place considerably more slowly than that of IgM but at the age of 1 year the infants with recurrent infections had however on the average more than twice as high IgA concentrations as the children in the BWC series On comparing this longitudinally studied series of infants in its entirety with the previous BWC series, which can be regarded as selected from the point of view of infection the former series was found to show a more rapid development of the IgG IgA and IgM levels during the first year of life

## ACKNOWLEDGEMENTS

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The author wishes to express his thanks to Ass Prof Claes Hogman for the facilities of his department placed at my disposal I also thank Ass Prof S Gunnar O Johansson for help with the immunoglobulin determinations Prof Gunnar Laurell for the bacteriological determinations my wife Ulla Berg R N for all her work with the collection of samples Mrs Birgit Landquist R N for help with the attempt at sterile nursing and Miss Birgitta Larsson Miss Lilian Posbilock Miss Britt Ytterberg and Mrs Ulla Zettersten for skilful technical assistance

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*Key words* Immunoglobulins infancy infections longitudinal studies

ned on the first occasion had been hydrolyzed by fungi or bacteria. Unfortunately this could never be checked.

*Quantitative assay of galactose in urine*

The quantitative assay of galactose in urine was performed with a method recently described by Tengstrom (39) in which the urine is filtered through a mixed ion exchange resin before quantitative assay with galactose oxidase (KABI AB Stockholm 30 Sweden). We used the anion exchanger in bicarbonate rather than hydroxyl form which seems to give better recovery.

The urine sample obtained on the 9th day after birth, shortly after the introduction of galactose free formula, contained 3400 mg% galactose (Fig. 1, Table 1). On galactose free diet the galactose content of the urine decreased during the course of 4 days to practically zero.

The reducing power of the urine samples assayed with the method of Somogyi-Nelson ( 9, 36) correlated well with the enzymatically assayed galactose content.

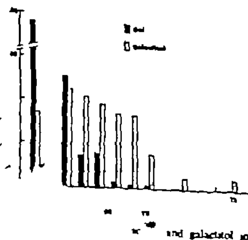
*Paper chromatography of sugars in urine*

Since the quantitative assay method for galactose is not quite specific but also lactose reacts when present in large amounts (9) the sugars present in the urine were also identified by paper chromatography. As solvent was used ethyl acetate acetic acid water (3 2 ?). With this solvent the urine can be applied to the paper without previous desalting. Whatman No. 1 paper was used and the chromatograms were run descendent for 18–20 hours. Staining was made with aniline phthalate (32). Only galactose was found in urine samples from the patient (Fig. 2).

*Assay of galactitol in urine*

In addition to galactose galactitol, i.e. the corresponding sugar alcohol formed on reduction of

The test paper in contrast seems to be more specific for galactose (8).



... and galactitol in ...

PATIENT MARKERS
Day 9 10

X X X X

lactose

galactose

glucose

*Fig. 2.* Paper chromatography of the urine samples obtained on the *9th and 10th days* (containing 3400 and 1200 mg/100 ml galactose, respectively) and two marker solutions (one containing *1000 mg/100 ml* galactose plus 1000 mg/100 ml glucose and the other one 2000 mg/100 ml galactose plus 1000 mg/100 ml lactose). Of each sample 5 µl was applied. The patient's urine contains only galactose and the intensity of the spots as compared with the marker fits well with the quantitative assay results.

galactose has been reported to be excreted in the urine of patients with galactosemia (31, 43). We therefore made attempts to identify galactitol in the urine of our patient by paper chromatography. In these experiments a periodate benzidine staining method (28) was used instead. Galactose and galactitol had, however, so similar rates of movement in all solvents tested that a final answer as to whether galactitol was present or not could not be obtained in this way. Therefore the galactose-galactitol spot in an unstained part of the chromatogram (located by parallel marker spots) was cut out, eluted with water

Table 1 Reaction of the urine with the galactose test paper

| Days after birth | Galactose in urine (mg/100 ml) | Test paper reaction Urine diluted 1 8 |
|---|---|---|
| 9 | 3400 | + + + |
| 10 | 1280 | + + + |
| 10½ | 370 | + + + |
| 11 | 399 | + + + |
| 11½ | 60 | (+) |
| 12 | 40 | (+) |
| 12½ | 30 | – |
| 13½ | 6 | – |
| 15 | 4 | – |

The urine has been tested after dilution 1 8 (which is the dilution used in screening of newborns for galactosuria with the test paper since a small amount of galactose can be found in the urine also from normal infants and this will react if the urine is analyzed without dilution (10)) Galactose free diet was introduced on the 9th day after birth

(glutamic pyruvic acid transaminase) 302 units at 11 days age with normal values ten days later Alkaline phosphatase activity in serum was normal the whole time The serum bilirubin reached a maximum value of 32 1 mg/100 ml on the 7th day of life before the first blood exchange transfusion All liver function tests were normal at 3 weeks of age

*EEG findings*[1]
At 10 days of age the EEG was pathological with frequent bursts of paroxysmal and partially epileptogenic activity appearing over both hemispheres Two weeks later the EEG showed a well organized background activity although still some suspect paroxysmal activity was left Normal EEG was found at later controls

*Clinical course*
After one month the patient returned home prescribed a diet of Sobee® and Galactomin® later supplemented with galactose free fruit and vegetable preparations The parents received instructions about the handling of the test paper for galactose in urine and regularly checked the patient's urine with this test paper

On one occasion at 3 / months of age moderate galactosuria was noted (see below) No clinical symptoms were noted on this occasion

At 5 / months of age the patient got an acute illness with vomiting diarrhea and abdominal distension His general condition became very bad within 2 days Laboratory investigation on this occasion did not demonstrate any galactose in blood or urine Liver tests were normal Fecal cultures did not reveal any pathogenic bacteria X ray examinations of the abdomen did not give any support for mechanical ileus Heart X ray showed general enlargement

Intravenous fluid therapy and large doses of supplementary vitamins were given The patient rapidly improved and could return home in good clinical condition at 6 months of age After this incident the diet was supplemented with Gevral® and folic acid

This episode reminds of reported cases of deficiency states in patients on synthetic foods (11 16) Our patient however did not show any pathological changes of the skin

Development has since then been satisfactory and at controls the latest one at 17 months of age the clinical and laboratory findings have been normal Length and weight developments appear normal and no neurologic sequelae have been found Psychologic testing at the latest control showed development equivalent to 12–13 months of age This is regarded as a slight retardation and concerns all functions equally

## SPECIAL INVESTIGATIONS

*Demonstration of galactose in urine with a specific test paper*
A recently described test paper for galactose in urine (7 8) reacting for galactose concentrations above 10–20 mg was strongly positive with a urine sample from the 9th day after birth and positive reaction was obtained even if the urine was diluted as much as 100 times with water indicating more than 1000 mg% galactose (compare the quantitative assay below) On galactose free diet the reaction of the urine with the test paper decreased in strength and after 4 days the reaction was negative also with undiluted urine (Table 1)

We have found it very convenient to use the test paper for control of the patient's urine Undiluted urine is used for these controls which are performed by the parents at home On one occasion the reaction became positive but no clinical symptoms were noted Quantitative analysis then showed the urine to contain 89 mg% galactose The galactose in this urine sample was also identified by paper chromatography The patient had on this occasion received Sobee® which does not contain free galactose but is known to contain galactose bound in certain oligosaccharides present in soya beans The presence of such oligosaccharides was verified by paper chromatography of the diet formula It has been reported that these galactose containing oligosaccharides are not digested or absorbed (13) On replacing Sobee® with Galactomin® the galactosuria promptly disappeared When later Sobee® was again introduced no galactosuria was found It is therefore possible that the galactose in the urine on the first occasion did not origin from Sobee® but from another source Another possibility is however that the oligosaccharides in the Sobee®

[1] The EEG registrations were analyzed at the clinical neurophysiological laboratory Sahlgren's Hospital Gothenburg

used on the first occasion had been hydrolyzed by fungi or bacteria. Unfortunately this could never be checked.

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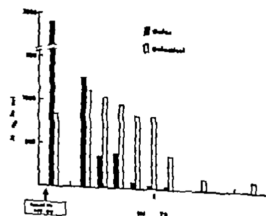


Fig 1 Concentration of galactose and galactitol in the urine from the patient.

PATIENT MARKERS
Day 9 10

X X X X

lactose

galactose

glucose

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Intravenous fluid therapy and large doses of supplementary vitamins were given The patient rapidly improved and could return home in good clinical condition at 6 months of age After this incident the diet was supplemented with Gevral® and folic acid

This episode reminds of reported cases of deficiency states in patients on synthetic foods (11 20) Our patient however did not show any pathological changes of the skin

Development has since then been satisfactory and at controls the latest one at 17 months of age the clinical and laboratory findings have been normal Length and weight developments appear normal and no neurologic sequelae have been found Psychologic testing at the latest control showed development equivalent to 12–13 months of age This is regarded as a slight retardation and concerns all functions equally

## SPECIAL INVESTIGATIONS

*Demonstration of galactose in urine with a specific test paper*

A recently described test paper for galactose in urine (7 8) reacting for galactose concentrations above 10–20 mg was strongly positive with a urine sample from the 9th day after birth and positive reaction was obtained even if the urine was diluted as much as 100 times with water indicating more than 1000 mg% galactose (compare the quantitative assay below) On galactose free diet the reaction of the urine with the test paper decreased in strength and after 4 days the reaction was negative also with undiluted urine (Table 1)

We have found it very convenient to use the test paper for control of the patient's urine Undiluted urine is used for these controls which are performed by the parents at home On one occasion the reaction became positive but no clinical symptoms were noted Quantitative analysis then showed the urine to contain 89 mg% galactose The galactose in this urine sample was also identified by paper chromatography The patient had on this occasion received Sobee® which does not contain free galactose but is known to contain galactose bound in certain oligosaccharides present in soya beans The presence of such oligosaccharides was verified by paper chromatography of the diet formula It has been reported that these galactose containing oligosaccharides are not digested or absorbed (13) On replacing Sobee® with Galactomin® the galactosuria promptly disappeared When later Sobee® was again introduced no galactosuria was found It is therefore possible that the galactose in the urine on the first occasion did not origin from Sobee® but from another source Another possibility is however that the oligosaccharides in the Sobee®

[1] The EEG registrations were analyzed at the clinical neurophysiological laboratory Sahlgren's Hospital Gothenburg

normal value which also was to be expected since the patient had recently undergone exchange blood transfusion three times. During the course of two months the galactose 1 phosphate uridyl transferase activity of the patient's blood decreased to a few units (Fig. 4). The enzyme activity disappeared with a half life of about 20 days which should be identical with the half life of the transfused erythrocytes. The patient's own erythrocytes therefore lacked galactose 1 phosphate uridyl transferase activity which definitively confirms the diagnosis of hereditary galactosemia. In a blood sample taken 6 months of age no activity could be found.

The galactose 1 phosphate uridyl transferase activity was assayed also in blood samples from the parents of the patient. The activities were 12 8 (father) and 12 1 (mother) units per g of hemoglobin. Thus both parents have values close to 50% of the normal activity which agrees with the activity previously reported in heterozygotes for this disease (2 14 21 4 35 40). This also fits with previous descriptions that galactosemia is recessively inherited (19 21). At a stage at which the analyses of blood from the patient could not verify the diagnosis because of the blood transfusions performed these values found in the parents gave strong support for the suspicion of hereditary galactosemia in the patient.

Our normal value for galactose 1 phosphate uridyl transferase activity in red blood cells is 26 9 ± 4 5 (S.D.) units per g hemoglobin.

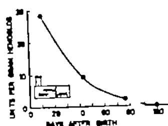



*Fig. 4.* Galactose 1 phosphate uridyl transferase activity in the red blood cells of the patient. Immediately after repeated exchange transfusions a normal value was found, but the activity then disappeared with a half life of about 20 days showing that the patient's own erythrocytes lack the enzyme. After 180 days no residual activity could be demonstrated.

Table 3. *Plasma concentration, urinary excretion and renal clearance of free amino acids 1 and 8 days after the beginning of the dietary treatment*

| | Plasma level (μ mole/l) | | Urinary excretion (μ mole/day) | | Renal clearance (l/day) | |
|---|---|---|---|---|---|---|
| Age (days) | 10 | 17 | 10 | 17 | 10 | 17 |
| Taurine | — | — | 733 | 5 | — | — |
| Aspartic acid | 40 | < 20 | 117 | 5 | — | — |
| Threonine | 214 | 110 | 778 | 21 | 3 6 | 0 2 |
| Serine | 572 | 156 | 964 | 7 | 1 7 | 0 1 |
| Glutamine | 1100 | 125 | 1170 | 10 | 1 1 | 0 1 |
| Proline | — | — | 260 | < 10 | — | — |
| Glutamic acid | 236 | 165 | 63 | 30 | 0 3 | 0 2 |
| Citrulline | — | — | 108 | 5 | — | — |
| Glycine | 806 | 191 | 29 0 | 176 | 3 6 | 0 9 |
| Alanine | 330 | 249 | 342 | 41 | 1 0 | 0 2 |
| Valine | 220 | 193 | 63 | 5 | 0 3 | 0 1 |
| Cystine | 0 | 20 | < 40 | 5 | — | — |
| Methionine | 54 | 40 | 20 | 7 | < 0 4 | 0 2 |
| Isoleucine | 74 | 64 | 34 | 5 | 0 5 | 0 2 |
| Leucine | 160 | 113 | 56 | < 5 | 0 4 | 0 1 |
| Tyrosine | 492 | 60 | 505 | 16 | 1 0 | 0 3 |
| Phenylalanine | 217 | 58 | 190 | 6 | 0 9 | 0 1 |
| Lysine | — | — | 172 | 52 | — | — |
| Ornithine | — | — | 72 | 10 | — | — |
| Histidine | — | — | 723 | 68 | — | — |
| Arginine | — | — | 15 | < 10 | — | — |

On the 2nd day of life the glutamine level was 550 μmol/l.

## DISCUSSION

In patients with hereditary galactosemia the enzyme galactose 1 phosphate uridyl transferase is absent as the result of an autosomal recessively inherited genetic defect. Thorough descriptions of the metabolism of galactose, the clinical findings in galactosemia and the variability of the clinical symptoms have been published (2 4 6 18 19 20 40 41) and these will therefore not be discussed here again.

The diagnosis of hereditary galactosemia is established much more rarely than the expected occurrence of the disease. Clinically 1 patient with hereditary galactosemia is found in 70 000 live births (2 3 35). Heterozygous carriers for the disease can be detected by quantitative assay of galactose 1 phosphate uridyl transferase activity in blood cells. Such studies have indicated the frequency of heterozygotes to be 1 50–1 67 corresponding to an expected frequency of the disease (homozygotes) of 1 10 000–1 18 000 (3 14). Early introduced dietary treatment is essential for the survival and normal development and therefore methods which can simplify the diagnosis in suspect cases—and especially methods

Table 2 *Galactose in serum samples assayed with galactose oxidase*

| Days after birth | Galactose in serum (mg/100 ml) |
|---|---|
| 5 | 150 |
| 9 | 137 |
| 9½[a] | 82 |
| 9½[b] | 38 |
| 10 | 1 |
| 11 | 6 |

Galactose free diet was introduced on the 9th day after birth

[a] Before 3rd exchange transfusion
[b] After 3rd exchange transfusion

and the concentration of sugar alcohol measured with the colorimetric method of Corcoran & Page (5) Galactose gives only a very weak reaction (0 5 % of the colour formed by the corresponding amount of galactitol) which could be corrected for With this method the urine samples obtained shortly after the exclusion of galactose from the diet of the patient were found to contain considerable amounts of galactitol in addition to galactose (Fig 1) On galactose free diet the galactitol content of the urine decreased to zero during the course of a few days but more slowly than its galactose content

*Assay of galactose in serum*

Galactose in serum was assayed with galactose oxidase (17 42) Serum blanks were run in order to correct for the interference of bilirubin The colour in these blanks corresponded to 5–15 mg/100 ml galactose in urine (Tables 1–3)

*Assay of galactose 1 phosphate in erythrocytes*

Galactose 1 phosphate was determined by the method of Kirkman and Maxwell (25) Normal blood was used as a source of uridyl transferase Samples obtained at 11–14 days and 6 months after birth did not contain any detectable amounts of galactose 1 phosphate This seems reasonable as the first samples were obtained after repeated exchange transfusions and the donor blood cells had normal galactose 1 phosphate uridyl transferase activity and would thus not accumulate galactose 1 phosphate even when galactose was administered On the second occasion the patient s blood contained his own uridyl transferase deficient erythrocytes but now he received since a long time a galactose free diet

*Assay of amino acids in plasma and urine*

Free amino acids in plasma and urine were estimated by ion exchange chromatography (37 38) and amino nitrogen as described by Ingenburg (23)

The urinary amino nitrogen excretion was excessively high but rapidly decreased after the dietary treatment was introduced (Fig 3) The amino aciduria was of generalized type (Table 3)

On the 10th day of life very high plasma levels were observed for some amino acids viz tyrosine serine glycine phenylalanine and threonine The levels of methionine glutamine and glutamic acid were moderately elevated whereas the levels of alanine and the branched chain amino acids were normal One week later the plasma amino acids were normalized except for a slight elevation of the methionine level The glutamine level as well as the urinary excretion of glutamine was very low at that time After a further five days the plasma glutamine level was normal

*Approximative renal amino acid clearance*

On the basis of the fasting plasma amino acid levels and the 24 hour amino acid excretion the approximative renal amino acid clearance was calculated (Table 3)

The amino aciduria was due not only to overflow but also to a defect renal tubular reabsorption After one week of dietary treatment the reabsorption had become normal

*Galactose 1 phosphate uridyl transferase activity in red blood cells*

In hereditary galactosemia galactose 1 phosphate uridyl transferase is missing which explains the metabolic defect (6 18 19 22) The defect can be demonstrated by measuring the enzyme activity in the erythrocytes We used the method of Tolstrup (7 40) with the following two modifications

1 The blood cells were washed twice with saline before the assay since the plasma in blood samples from infants sometimes has been found to interfere with the assay (to be published)

2 The hemolysate was preincubated with dithiothreitol 5 mg/ml for 15 minutes prior to the assay of enzyme activity Dithiothreitol has been found to protect the enzyme activity and reactivate enzyme that may have been oxidized since galactose 1 phosphate uridyl transferase is a sulfhydryl enzyme (7)

Blood from the patient obtained at 10 days of age showed galactose 1 phosphate uridyl transferase activity of 27 0 units per gram hemoglobin This is a

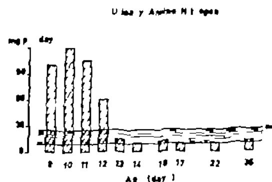


Fig 3 Urinary amino nitrogen excretion Galactose free diet was introduced on the 9th day after birth

normal value which also was to be expected since the patient had recently undergone exchange blood transfusion three times. During the course of two months the galactose 1 phosphate uridyl transferase activity of the patient's blood decreased to a few units (Fig. 4). The enzyme activity disappeared with a half life of about 20 days which should be identical with the half life of the transfused erythrocytes. The patient's own erythrocytes therefore lacked galactose 1 phosphate uridyl transferase activity which definitively confirms the diagnosis of hereditary galactosemia. In a blood sample taken 6 months of age no activity could be found.

The galactose 1 phosphate uridyl transferase activity was assayed also in blood samples from the parents of the patient. The activities were 12.8 (father) and 1 .1 (mother) units per g of hemoglobin. Thus both parents have values close to 50 % of the normal activity which agrees with the activity previously reported in heterozygotes for this disease (2 14 21 24 35 40). This also fits with previous descriptions that galactosemia is recessively inherited (19

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Our normal value for galactose 1 phosphate uridyl transferase activity in red blood cells is 26.9 ± 4.5 (S.D.) units per g hemoglobin.

Table 3. *Plasma concentration, urinary excretion and renal clearance of free amino acids 10 and 17 days after the beginning of the dietary treatment*

| | Plasma level (μmole/l) | | Urinary excretion (μmole/day) | | Renal clearance (l/day) | |
|---|---|---|---|---|---|---|
| Age (days) | 10 | 17 | 10 | 17 | 10 | 17 |
| Taurine | — | — | 733 | < 5 | — | — |
| Aspartic acid | 70 | 70 | 117 | < 5 | — | — |
| Threonine | 214 | 110 | 778 | 21 | 3.6 | 0.2 |
| Serine | 572 | 156 | 964 | 7 | 1.7 | 0.1 |
| Glutamine | 1100 | 125 | 1170 | 10 | 1.1 | 0.1 |
| Proline | — | — | 260 | < 10 | — | — |
| Glutamic acid | 436 | 165 | 63 | 30 | 0.3 | 0.2 |
| Citrulline | — | — | 108 | 5 | — | — |
| Glycine | 806 | 191 | 2970 | 176 | 3.6 | 0.9 |
| Alanine | 330 | 249 | 342 | 41 | 1.0 | 0.2 |
| Valine | 270 | 193 | 63 | 5 | 0.3 | 0.1 |
| Cystine | 0 | 20 | 40 | 5 | — | — |
| Methionine | 54 | 40 | 20 | 7 | 0.4 | 0.2 |
| Isoleucine | 74 | 64 | 34 | 5 | 0.5 | 0.2 |
| Leucine | 160 | 113 | 56 | 5 | 0.4 | 0.1 |
| Tyrosine | 492 | 60 | 505 | 16 | 1.0 | 0.3 |
| Phenylalanine | 17 | 58 | 190 | 6 | 0.9 | 0.1 |
| Lysine | — | — | 177 | 52 | — | — |
| Ornithine | — | — | 7 | 18 | — | — |
| Histidine | — | — | 723 | 68 | — | — |
| Arginine | — | — | 15 | 10 | — | — |

On the [illegible] day of life the glutamine level was 530 μmol/l.

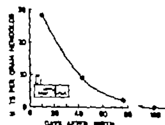



*Fig. 4.* Galactose 1 phosphate uridyl transferase activity in the red blood cells of the patient. Immediately after repeated exchange transfusions a normal value was found but the activity then disappeared with a half life of about 20 days showing that the patient's own erythrocytes lack the enzyme. After 180 days no residual activity could be demonstrated.

21). At a stage at which the analysis of blood from the patient could not verify the diagnosis because of the blood transfusions performed these values found in the parents gave strong support for the suspicion of hereditary galactosemia in the patient.

## DISCUSSION

In patients with hereditary galactosemia the enzyme galactose 1 phosphate uridyl transferase is absent, as the result of an autosomal recessively inherited genetic defect. Thorough descriptions of the metabolism of galactose the clinical findings in galactosemia and the variability of the clinical symptoms have been published (2 4 6 18 19 20 40 41) and these will therefore not be discussed here again.

The diagnosis of hereditary galactosemia is established much more rarely than the expected occurrence of the disease. Clinically 1 patient with hereditary galactosemia is found in 70 000 live births (2 3 35). Heterozygous carriers for the disease can be detected by quantitative assay of galactose 1 phosphate uridyl transferase activity in blood cells. Such studies have indicated the frequency of heterozygotes to be 1 50–1 67 corresponding to an expected frequency of the disease (homozygotes) of 1 10 000–1 18 000 (3 14). Early introduced dietary treatment is essential for the survival and normal development and therefore methods which can simplify the diagnosis in suspect cases—and especially methods

which are simple enough for screening purposes—are badly needed One of the reasons for which we want to report our case is because it is the first patient which has been studied with a recently developed galactose specific test paper for urine which seems to be a very promising aid for the diagnosis of galactosemia

The clinical symptoms of our patient agreed well with the classical symptoms of hereditary galactosemia (6 19) The demonstration of the lack of galactose 1-phosphate uridyl transferase activity gave unequivocal proof of the diagnosis Since galactose free diet was introduced already on the 9th day after birth the prognosis should be good if this diet is strictly followed (4) Although the patient was in a very bad condition when the diagnosis was established he rapidly improved on dietary treatment The fact that exchange blood transfusion had been performed several times before the diagnosis of galactosemia was considered in our patient caused a difficulty in that the diagnosis could not be tested immediately by enzyme assay in a blood sample The extremely high levels of galactose found in urine, and the rapid improvement on galactose free diet made however the diagnosis highly probable already at this stage A further indication of the correctness of the diagnosis could also be obtained immediately by analysis of the enzyme activity in the blood from the parents which showed heterozygote values First when the transfused blood corpuscles had essentially disappeared could the diagnosis be definitively proven with a blood sample from the patient

Our patient reacted positively in the PKU screening test, which seems to be a reflection of liver damage Several amino acids were present in much higher concentration than normal in the blood These changes were quite reversible and normalized dramatically on dietary treatment as well as other symptoms of impaired liver function

The high concentration of amino acids in the urine may partly be a reflection of the high blood values but the clearance studies showed that also a kidney damage with impaired tubular reabsorption was involved Thus both the liver and the kidneys are damaged by galactose ingestion

Extremely high galactose concentration was found in the urine samples collected from our patient shortly after the introduction of galactosefree diet The positive reducing sugar test noted at six days of age indicates that the patient had a massive galactosuria already then The galactose concentration in the urine of our patient seems very promising for the possibilities to use the galactose specific urine test paper as a screening method for hereditary galactosemia Further investigation will however, *have to be performed in order* to establish the normal limits and pilot studies are now being performed In earlier publications only few figures are available for the galactose content of urine from untreated galactosemia patients, mainly because suitable methods of analysis have not been available Bickel (1) stated that *urine from such patients* contained over 400 mg/100 ml galactose, as judged from semi quantitative paper chromatography and this is a sufficiently high figure to assure a strong test paper reaction Zetterstrom (44) in a pair of twins with untreated galactosemia found 1500 mg/100 ml galactose in the urine on the 4th day *after birth* using a reducing sugar method for the assay Hessel *et al* (16) in an 11 days old patient found 5000 mg/100 ml galactose in the urine by polarimetry It has been proposed that relatively low galactose excretion in the urine from patients with galactosemia may occur if the patient vomits and fails to eat Our patient had however started to vomit and refuse eating already before the urine samples with high galactose content were obtained

Considerable amounts of galactitol also were excreted with the urine and the excretion of this sugar alcohol persisted longer than that of galactose which is in agreement with results recently published by Wells Pittman & Egan (43) Assay of galactitol in the urine may therefore become a helpful complementary method in the diagnosis of galactosemia

A continued control of that the patient receives a completely galactose free diet is highly desirable. It has been shown that such a low level of galactose in the diet as 200 mg is sufficient to cause an accumulation of galactose 1 phosphate in the blood cells of galactosemia patients (41). The methods available for assay of galactose 1 phosphate are however relatively complicated and only few laboratories perform these assays (25, 34). It therefore seems to be a valuable application of the galactose test paper to use it for the control of the patient's urine. In our patient this control gave a positive reaction on one occasion at which galactose was excreted in the urine before any clinical symptoms were noted. This control therefore seems to be very sensitive and well suited for checking the patient's diet. It is so simple that in many cases the parents will be able to perform this control themselves at regular intervals. Further research is however indicated in order to establish the relations between dietary galactose, accumulation of galactose 1 phosphate in the erythrocytes, and excretion of galactose in the urine.

## SUMMARY

A case of hereditary galactosemia in a newborn infant is reported. Dietary treatment was started on the 9th day of life which caused a dramatical improvement in the patient's condition. Diagnosis was confirmed by assay of galactose in urine and blood and finally by demonstration of the enzyme deficiency in the red blood cells of the patient. Both parents had low values for galactose 1 phosphate uridyl transferase activity in red blood cells in agreement with the fact that they are heterozygotes for hereditary galactosemia.

A recently described test paper for galactose in urine was used both in the diagnosis of the disease and for the control of the suitability of the patient's diet.

In addition to galactose the urine also contained large amounts of galactitol. On the introduction of galactose free diet galactitol disappeared from the urine more slowly than did galactose.

There was an increased concentration of amino acids in the blood and in the urine as a result of liver damage and also decreased tubular reabsorption. Also the aminoaciduria and the increased blood amino acid concentration disappeared on galactose free diet.

## ACKNOWLEDGMENT

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# AMINO ACID PATTERNS IN PLASMA AND ERYTHROCYTES IN PROTEIN MALNUTRITION

## C N U Report 13

K B BJÖRNESJÖ R JAGENBURG and O MELLANDER

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Alterations of the plasma amino acid pattern in protein malnutrion have been reported from several parts of the world (2 7 10 15 16) Most of the investigators have found a de crease especially of the essential amino acids valine leucine isoleucine and tyrosine but normal values have also been reported (11) The changes observed in the amino acid pat tern seem to occur earlier than other biochemi cal signs in protein malnutrition and much attention has therefore been paid to this subject during the last few years Unfortunately a com plete quantitative amino acid analysis is still too time consuming to be used routinely in field work Whithead (14) for that reason introduced a simple paper chromatographic screening test of the plasma amino acid pat tern Whitehead suggested the G/VL ratio ob tained in this test as a useful index of amino acid imbalance In this ratio G indicates the sum of the absorbance values for the amino acids glycine glutamine and serine and VL the sum of the absorbance values for valine leucine and isoleucine

It seems possible that additional information can be obtained by analyses of intracellular free amino acids The red cell amino acids are not always correlated to the plasma levels (3 6) In protein malnutrition normal or even in creased values of some amino acids have been observed in red cells in spite of very low plasma values (8 9)

The purpose of the present investigation was (1) to investigate the reliability of the screening test by comparison with quantitative column chromatography (2) to use the screen ing technique in a field study of protein mal nutrition among Ethiopian pre school children and for comparison in apparently healthy chil dren and children with advanced protein mal nutrition, and (3) to study the erythrocyte/ plasma distribution ratios of amino acids in protein malnutrition

## MATERIAL

Amino acid screening has been performed on blood serum from 711 pre-school children from the Ethio-Swedish Children s Nutrition Unit field stations in Addis Ababa, Ijaji and Sadamo and on blood plasma and erythrocyte haemolysate from 44 children 3–36 months of age with advanced protein malnutrition admitted to the Ethio Swedish Pediatric Clinic in Ad dis Ababa The group of advanced protein malnutri tion includes both kwashiorkor and marasmic cases All of the kwashiorkor cases showed growth retarda tion and muscle wasting with retention of some sub cutaneous fat. Oedema and psychomotor changes were present in all cases The marasmic cases showed extreme growth retardation and wasting of muscle and subcutaneous fat. Similar changes in red cell and plasma amino acid pattern were found in both groups. All cases listed in Table 2 were of the

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# AMINO ACID PATTERNS IN PLASMA AND ERYTHROCYTES IN PROTEIN MALNUTRITION

C N U Report 13

K. B BJÖRNESJÖ R. JAGENBURG and O MELLANDER

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Alterations of the plasma amino acid pattern in protein malnutrition have been reported from several parts of the world (2 7 10 15 16) Most of the investigators have found a decrease especially of the essential amino acids valine leucine isoleucine and tyrosine but normal values have also been reported (11) The changes observed in the amino acid pattern seem to occur earlier than other biochemical items in protein malnutrition and much attention has therefore been paid to this subject during the last few years Unfortunately a complete quantitative amino acid analysis is still too time consuming to be used routinely in field work Whitehead (14) for that reason introduced a simple paper chromatographic screening test of the plasma amino acid pattern Whitehead suggested the G/VL ratio obtained in this test as a useful index of amino acid imbalance In this ratio G indicates the sum of the absorbance values for the amino acids glycine glutamine and serine and VL the sum of the absorbance values for valine leucine and isoleucine

It seems possible that additional information can be obtained by analysis of intracellular free amino acids The red cell amino acids are not always correlated to the plasma levels (3 6) In protein malnutrition normal or even increased values of some amino acids have been observed in red cells in spite of very low plasma values (8 9)

The purpose of the present investigation was (1) to investigate the reliability of the screening test by comparison with quantitative column chromatography (2) to use the screening technique in a field study of protein malnutrition among Ethiopian pre school children and for comparison in apparently healthy children and children with advanced protein malnutrition and (3) to study the erythrocyte/plasma distribution ratios of amino acids in protein malnutrition

## MATERIAL

Amino acid screening has been performed on blood serum from 711 pre-school children from the Ethio-Swedish Children's Nutrition Unit field stations in Addis Ababa Lipp, and Sidamo and on blood plasma and erythrocyte haemolysate from 44 children 3–36 months of age with advanced protein malnutrition admitted to the Ethio-Swedish Pediatric Clinic in Addis Ababa. The group of advanced protein malnutrition includes both kwashiorkor and marasmic cases All of the kwashiorkor cases showed growth retardation and muscle wasting with retention of some subcutaneous fat. Oedema and psychomotor changes were present in all cases The marasmic cases showed extreme growth retardation and wasting of muscle and subcutaneous fat Similar changes in red cell and plasma amino acid pattern were found in both groups. All cases listed in Table 2 were of th

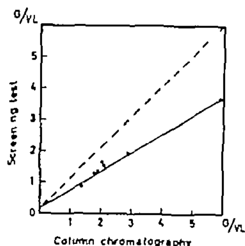



*Fig 1* Correlation between G/VL ratios obtained in the screening test and in quantitative column chromatography ($r = 0.94$ S.D. $= 0.022$) Dashed line represents molar G/VL ratio

Kwashiorkor type For comparison fasting and non fasting blood samples from 42 healthy Ethiopian children of corresponding age from an orphanage in Addis Ababa was studied Quantitative column chromatography of amino acids was performed on some of the blood samples to test the reliability of the screening method

## METHODS

Amino acid screening was performed according to Whitehead (14) with minor modifications of the original technique (7) The erythrocyte haemolysates were prepared as follows The red cells from 5 ml blood were washed twice with 3 ml aliquots of saline Two rapid washings did not give significant loss of amino acids from the erythrocytes The red cells were haemolyzed by adding two volumes of distilled water and freezing By haematocrit determination of the concentrated suspensions of red cells it was possible to relate the volume of haemolysate to the original volume of red cells Aliquots of the haemolysate corresponding to 0 1 ml of red cells were transferred to tubes containing 4 ml 95 (v/v) ethanol for precipitation of proteins For protein precipitation of plasma 4 ml of 90 % (v/v) ethanol was added to 0 1 ml plasma The ethanol extracts were evaporated over a boiling water bath To ensure as low a temperature as possible a stream of air was blown over the sample The dry residues were dissolved each in 0 2 ml 10 % (v/v) isopropyl alcohol and applied quantitatively to the chromatography paper Plasma and erythrocyte samples obtained from the same blood were always run on the same paper A control serum (freeze stored in small ampoules) was included in each run After the run the papers were allowed to dry at room temperature and then stained in consecutive solutions of 0 2 % (w/v) ninhydrin in acetone and copper nitrate in acid ethanol (1 ml saturated copper nitrate in water 100 ml 96 (v/v) ethanol and 0 2 ml 10 % (w/v) nitric acid) A group separation of the amino acids was obtained according to the following G spot (glycine + glutamine + serine) T spot (threonine + glutamic acid) A spot (alanine) V spot (valine + methionine) and L spot (leucine + isoleucine) The other plasma and erythrocyte amino acids normally give faint spots which were unsuitable for quantitative evaluation The spot caused by erythrocyte glutathione was located near the starting point well separated from the amino acids Simple viewing of the chromatograms after staining gave an approximate idea of the distribution pattern For quantitative evaluation the spots were extracted with 4 ml aliquots of methanol and the extracts read in a photometer at a wave length of 510 $\mu m$ Besides the G/VL ratio the A/L ratio was calculated routinely Minor variations in the technique will not influence the distribution ratios for they will affect the plasma and red cell amino acids to the same extent When calculating the distribution ratios for the amino acids the amounts in an equal volume (0 1 ml) of red cells and plasma were compared This ratio can be transferred to the distribution ratio erythrocyte/plasma water by multiplying by a factor of 1 4? assuming that red cells have a water content of 65 % and plasma 92 % (1)

Amino nitrogen of plasma and red cells has been determined as described by Bjornesjo (6) For quantitative determination of individual free amino acids the method of Spackman *et al* (12) was used Deproteinization was performed with picric acid (13)

### *Reliability of the amino acid screening technique*

Increasing quantities (up to 20 $\mu g$) of amino acid applied to the paper gave after chromatography a rectilinear increase in absorbance values The reproducibility of the method was satisfactory (Table 1) The correlation between the screening technique and the quantitative column chromatography was also satisfactory so far as the G/VL ratio was concerned (Fig 1) but the levels of the ratio differ because the amino acids evaluated in the screening test have different molar absorbance values However the molar absorb-

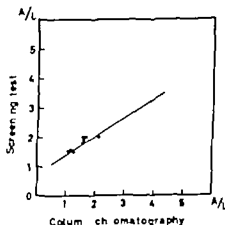



*Fig 2* Correlation between A/L ratios obtained in the screening test and in quantitative column chromatography ($r = 0.75$ S.D. $= 0.087$)

ance values for valine leucine and isoleucine are identical and the molar absorbance values for the amino acids in the glycine group are very similar The methionine values are so low that they can be disregarded It is thus possible to correct the G/VL ratio obtained in the screening test to molar G/VL ratio After this correction similar ratios are obtained with the two techniques The corresponding correlation for the A/L ratio was less satisfactory (Fig 2)

The erythrocyte absorbance values for valine and leucine ± isoleucine obtained by the screening technique were in good agreement with the quantities in micromolar per gram erythrocyte obtained by column chromatography (Figs 3–4)

*Plasma amino acids and food intake*

The plasma amino acid levels and thus also the G/VL and A/L ratios can be influenced by food intake

However the average difference between the G/VL and A/L ratios for the fasting and non fasting control groups was insignificant The standard deviation was slightly higher in the non fasting group The normal range in Figs 5 and 6 is M ± 2 s D for the combined material In the field study it was not possible to obtain fasting blood samples from all children but according to enquiries it is most unlikely that these children have obtained animal protein in quantities which can be assumed to change the G/VL or A/L ratios significantly

## RESULTS AND DISCUSSION

*G VL and A/L serum ratios in Ethiopian pre school children and in children with advanced protein malnutrition*

The G/VL ratios were increased in 28 27 and 45 respectively in the serum specimens

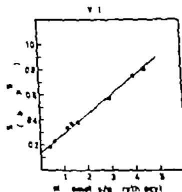


Fig 3 Valine in erythrocytes. Correlation between screening test absorbance values and micromoles per gram erythrocyte obtained by quantitative column chromatography (r = 0 985 s.D = 0 009)

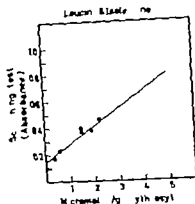


Fig 4 Leucine + isoleucine in erythrocytes. Correlation between screening test absorbance values and micromoles per gram erythrocyte obtained by quantitative column chromatography (r = 0 915 s D = 0 05)

from the field stations in Addis Ababa Jjap and Sidamo (Fig 5) The corresponding figures for the A/L ratios were 14 17 and 52 (Fig 6) The high frequency of increased G/VL and A/L ratios suggests inadequate protein supply among the pre school children in these areas The very high frequency of amino acid imbalance among the children at the Sidamo station is of special interest In this area the ensete (false banana *Ensete edulis*) which has a very low protein content is the main staple food

For comparison the G/VL and the A/L plasma ratios have been determined in children with advanced protein malnutrition (Figs 5–6) The G/VL ratios are increased in most cases The A/L ratios show remarkable variability due to considerable variation in the alanine values Subnormal A/L ratios are often found due to very low alanine values (Fig. 6) The high incidence of increased A/L ratios among the children in the Sidamo area, however suggests that the A/L ratios may also be of value as a complement to the G/VL ratios in protein malnutrition studies Despite the fact that amino acid imbalance with high G/VL ratios seems to be an early sign of protein malnutrition (15 16) several of our advanced cases have normal G/VL ratios (Fig. 5) This fact may have the same explanation as the high

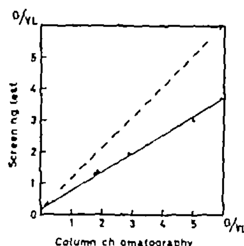



*Fig 1* Correlation between G/VL ratios obtained in the screening test and in quantitative column chromatography ($r = 0.94$ S D $= 0.072$) Dashed line represents molar G/VL ratio

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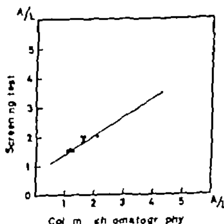



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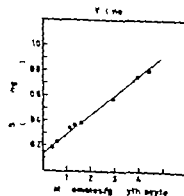


Fig 3. Valine in erythrocytes. Correlation between screening test absorbance values and micromoles per gram erythrocyte obtained by quantitative column chromatography (r = 0.994, s.D. = 0.009).

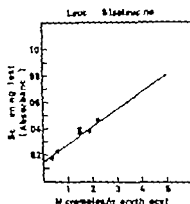


Fig 4. Leucine + isoleucine in erythrocytes. Correlation between screening test absorbance values and micromoles per gram erythrocyte obtained by quantitative column chromatography (r = 0.915, s.D. = 0.05).

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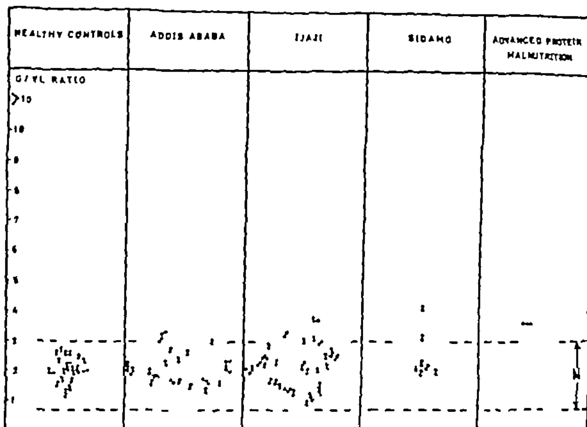



*Fig 5* G/VL serum ratios for children from the CNU field stations in Addis Ababa ($n=290$) Ijaji ($n=311$) and Sidamo ($n=110$) compared to children with advanced protein malnutrition ($n=81$) and to healthy controls ($n=84$ $N$=normal range mean ± 2×S D)

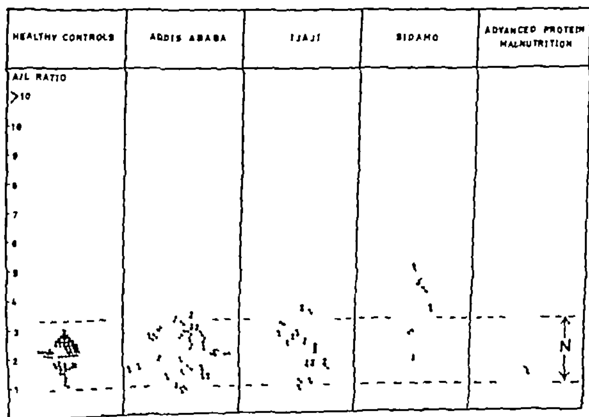



*Fig 6* A/L serum ratios for children from the CNU field stations in Addis Ababa ($n=290$) Ijaji ($n=230$) and Sidamo ($n=110$) compared to children with advanced protein malnutrition ($n=81$) and to healthy controls ($n=84$ $N$=normal range mean ±2×S D

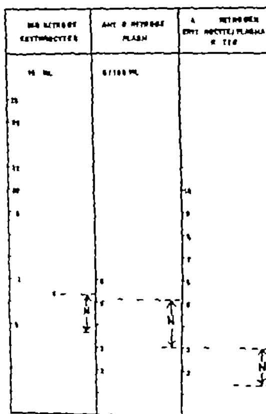


Fig 7 Distribution of alpha amino nitrogen between erythrocytes and plasma in 34 cases of protein malnutrition compared to healthy controls (n=28 N = normal range mean ±2 SD)

values of serum urea nitrogen and alpha amino nitrogen which sometimes occur in severe protein malnutrition i e increased breakdown of tissue proteins when other sources of calories are lacking (7)

*Erythrocyte plasma distribution ration of amino acids in advanced protein malnutrition*

The normal distribution ratios between volume unit red cells and plasma are in the range of 1–2 for most amino acids Analysis of alpha amino nitrogen content of red cells and plasma in the children hospitalized for protein calorie malnutrition gave the surprising result that the amino nitrogen content of the red cells was often increased despite low plasma values (Fig

Table 1 *Reproducibility of absorbance values and G/VL and A/L ratios by 30 consecutive analysis of the control serum by the screening technique*

| Spot | | Mean | ±SD | Variation coefficient |
|---|---|---|---|---|
| G | Glycine + glutamine + serine | 0 446 | 0 039 | 8 8 |
| T | Threonine + glutamic acid | 0 144 | 0 010 | 7 0 |
| A | Alanine | 0 141 | 0 010 | 7 1 |
| V | Valine | 0 110 | 0 012 | 10 9 |
| L | Leucine + isoleucine | 0 094 | 0 010 | 10 7 |
| | G/VL | 2 15 | 0 12 | 5 6 |
| | A/L | 1 48 | 0 071 | 4 8 |

7) Paper chromatographic comparison of the erythrocyte and plasma content of amino acids showed that many amino acids were increased in the red cells High erythrocyte values of valine and leucine isoleucine were often found in spite of extremely low plasma values resulting in very high distribution ratios of these

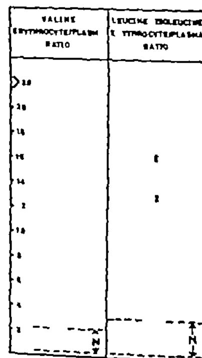


Fig 8 Erythrocyte/plasma ratios for valine and leucine + isoleucine in 44 cases of protein malnutrition compared to healthy controls (n=28 N = normal range mean ±2 SD)

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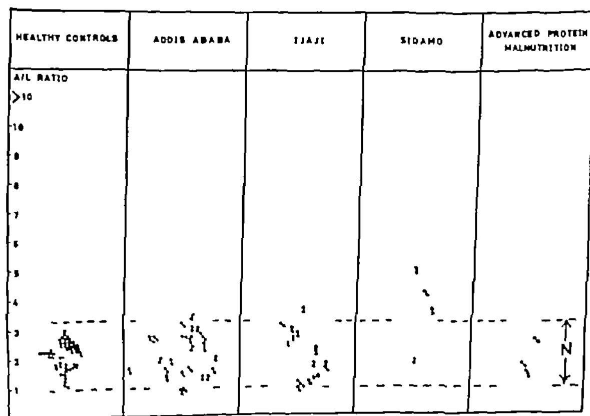



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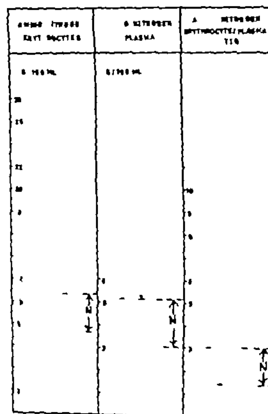


Fig 7 Distribution of alpha amino nitrogen between erythrocytes and plasma in 34 cases of protein malnutrition compared to healthy controls (n = 28 N = normal range mean ± SD)

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| L Leucine + isoleucine | 0 094 | 0 010 | 10 7 |
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| A/L | 1 48 | 0 071 | 4 8 |

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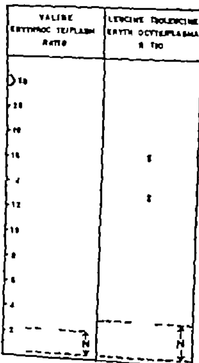


Fig 8 Erythrocyte/plasma ratios for valine and leucine + isoleucine in 44 cases of protein malnutrition compared to healthy controls (n = 28 N = normal range mean ± 2 × SD)

Table 2 *Erythrocyte/plasma ratios for valine and isoleucine leucine in advanced protein malnutrition (Kwashiorkor) before (I) and after (II) treatment*

| Patient no | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Days of treatment | 25 | 22 | 33 | 15 | 20 | 6 |
| Valine | | | | | | |
| I | 36 6 | 15 0 | 15 3 | 15 0 | 12 8 | 3 3 |
| II | 7 2 | 1 1 | 2 6 | 3 7 | 2 6 | 2 9 |
| Isoleucine leucine | | | | | | |
| I | 46 7 | 5 1 | 7 6 | 16 2 | 65 5 | 3 8 |
| II | 6 2 | 0 9 | 3 0 | 5 1 | 3 8 | 3 4 |

amino acids (Fig 8) The high distribution ratios decrease rapidly after successful treatment (Table 2)

Increase in the erythrocyte/plasma distribution ratios of amino acids is not specific for protein malnutrition It has been found also in other conditions affecting protein metabolism e g in pregnancy malignant disease and active inflammation (4 5) However the distribution ratios in protein malnutrition are mostly higher due to the low plasma amino acid content

## SUMMARY

The reliability of the paper chromatographic technique developed by Whitehead for evaluation of the amino pattern of serum and plasma has been studied by comparison with the results obtained by quantitative column chromatography The results obtained with the two techniques show a satisfactory agreement

The screening technique has been used in a field study of protein malnutrition among Ethiopian pre school children in different areas of the country Changes of the amino acid pattern of blood serum of the type seen in protein malnutrition have been found in 27–52 of the children The highest figure of amino acid imbalance was obtained for an area where ensete (false banana) containing only about 1 of protein is the main staple food

The erythrocyte/plasma distribution ratios of amino acids have been determined in a group of children with advanced protein malnutrition The erythrocyte values of amino acids are often increased in spite of low plasma values resulting in high erythrocyte/plasma distribution ratios in most of the protein malnutrition cases studied

## ACKNOWLEDGEMENT

The authors want to express their appreciation to Gunnar Arhammar M D for his help in obtaining blood specimens from patients at the Ethio Swedish Pediatric clinic and to Ato Makonnen Belew PhD for participating in the amino acid analyses of part of the material

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Key words Amino acid patterns erythrocytes plasma paper chromatography column chromatography pre school children malnutrition

Table 2 *Erythrocyte/plasma ratios for valine and isoleucine leucine in advanced protein malnutrition (kwashiorkor) before (I) and after (II) treatment*

| Patient no | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Days of treatment | 25 | 22 | 33 | 15 | 20 | 6 |
| Valine | | | | | | |
| I | 36 6 | 15 0 | 15 3 | 15 0 | 12 8 | 3 3 |
| II | 7 2 | 1 1 | 2 6 | 3 7 | 2 6 | 2 9 |
| Isoleucine leucine | | | | | | |
| I | 46 7 | 5 1 | 7 6 | 16 2 | 65 5 | 3 8 |
| II | 6 2 | 0 9 | 3 0 | 5 1 | 3 8 | 3 4 |

amino acids (Fig 8) The high distribution ratios decrease rapidly after successful treatment (Table 2)

Increase in the erythrocyte/plasma distribution ratios of amino acids is not specific for protein malnutrition It has been found also in other conditions affecting protein metabolism e g in pregnancy malignant disease and active inflammation (4 5) However the distribution ratios in protein malnutrition are mostly higher due to the low plasma amino acid content

## SUMMARY

The reliability of the paper chromatographic technique developed by Whitehead for evaluation of the amino pattern of serum and plasma has been studied by comparison with the results obtained by quantitative column chromatography The results obtained with the two techniques show a satisfactory agreement

The screening technique has been used in a field study of protein malnutrition among Ethiopian pre school children in different areas of the country Changes of the amino acid pattern of blood serum of the type seen in protein malnutrition have been found in 27–52 of the children The highest figure of amino acid imbalance was obtained for an area where ensete (false banana) containing only about 1 of protein is the main staple food

The erythrocyte/plasma distribution ratios of amino acids have been determined in a group of children with advanced protein malnutrition The erythrocyte values of amino acids are often increased in spite of low plasma values resulting in high erythrocyte/plasma distribution ratios in most of the protein malnutrition cases studied

## ACKNOWLEDGEMENT

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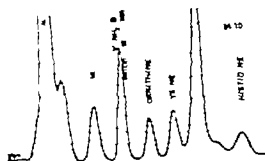


Fig 1 Chart No 84 10 Chromatogram showing the basic amino acids studied in 300 µl of plasma of an early born baby at one week of age Gamma amino buture acid was added to the sample as internal standard for colour yield

and lyophilized in order to remove ammonia and then readjusted to pH 2 0-2

A modified system of ion exchange chromatography was worked out in order to achieve reproducible determinations of essential amino acid levels on small amounts of plasma Tryptophan which is partly bound to plasma albumin (19) and is lost upon deproteinization and deprotenization (29) and threonine which is overlapped by serine and glutamine on chromatography using sodium citrate buffers were excluded in this study Histidine which is an essential amino acid during growth and tyrosine which is essential to the foetus due to the lack of phenylalanine hydroxylase activity in the liver of foetuses (13) were included in the study

The equipment consisted of two Beckman Accu Flo pumps an LKB multichannel absorptiometer Type 4950 B fitted with 3 mm cuvettes and Philips 1 channel recorder PR 4069 m 04 with two fold linear amplification The temperature of the column was kept constant by means of a Papst Colora thermostate K 4000 and the water bath by means of an Isopad Isomantle at 700 V The composition of the buffers and ninhydrine solution was that described by Spackman *et al* (28)

To 300 µl of plasma were added 1.5 ml of 1.2 picric acid The precipitation was removed by centrifugation at room temperature and an aliquote of 1 5 ml withdrawn lyophilized and stored at −20 C. The dry sample was dissolved in 270 µl of 0 1 − N HCl excess picric acid was removed by centrifugation at room temperature 200 µl of the supernatant plus 25 µl of 1 15 $10^{-4}$ − *N* γ aminobutyric acid as internal standard were loaded on the column

The basic amino acids were determined as follows The column size was 3×300 mm and Zeolite 225 (NC 8 % spherical particles) was used as resin over a combined teflon + steel filter After each analysis the resin was washed with 1 ml of 0 2 − N NaOH containing 0 67 g of BRIJ 35/liter and then regenerated with 1 ml of the pH 4 26 buffer The tube volume between the pump and the column plus the space above the resin was 4 3 ml After loading the sample and filling the space above the resin with pH 4 6 buffer the pH 5 28 buffer was pumped through the system at a flow rate of 8 0 ml/hour The flow rate of the ninhydrine solution was 4 0 ml/hour The temperature was kept at 27 2 C Picric acid emerged as a sharp peak after and overlapping the acidic and neutral amino acid but left the rest of the chromatogram as well resolved peaks (Fig 1) Histidine was eluted after 210 minutes and arginine emerged as a broad flat peak after a further 120 minutes. Temperature flow rate and volume of the pH 4 76 buffer were very critical and small alterations gave a poor resolution or a bad reproducibility Contamination of the buffers with ammonia easily gave a plateau which made integration of lysine and histidine peaks difficult. The integration was made by the height times width method

The neutral amino acids were determined according to the following modifications The column size was 3 650 mm and Beckman resin AA 15 was used which gave a better resolution of the neutral amino acids than the Zeolite previously used The column was washed with 4 ml of 0 2 − N NaOH + BRIJ 0.67

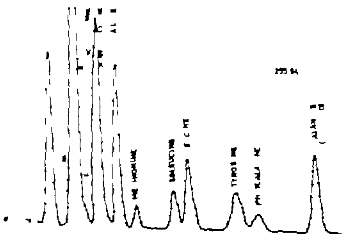


Fig Chart No 255 94 Chromatogram showing the neutral amino acids studied in 300 µl of plasma of a full term baby at 15 days of age Beta alanine was added to the sample as internal standard for colour yield

*Acta Pædiat Scand* 58 252–260 1969

# TIME STUDIES ON FREE AMINO ACID LEVELS OF VENOUS PLASMA DURING THE NEONATAL PERIOD

B S LINDBLAD and A BALDESTEN

*From the Department of Paediatrics at Crown Princess Lovisa s Children s Hospital and the Department of Chemistry II Karolinska Institutet Stockholm Sweden*

The aminogram of human cord vein plasma is characterized by hyperaminoacidemia with a marked increase of the lysine and taurine levels (15) The high levels decline towards the normal levels of infancy (27) about a week after birth (11 23) The plasma levels of tyrosine and proline are known to stay high for a longer period (4 11 14)

Studies of the plasma amino acid homeostasis in the neonatal period could give valuable human correlation to the knowledge of foetal and neonatal protein metabolism in animals The need for early screening tests of inborn errors of amino acid metabolism makes such studies necessary

A method of ion exchange chromatography on small amounts of human plasma has therefore been worked out and has been applied in a survey of venous plasma free amino acid levels of the normal human full term and early born during the neonatal period

## EXPERIMENTAL

The material is based on 9 newborns 5 full term and 4 early born They were all taken after an uneventful normal delivery to an incubator in a newborn unit within one hour of delivery They showed temperatures >35 C upon arrival at the incubator There had been no signs of intrauterine asphyxia and the Apgar s score had been 10 at 4 minutes after delivery In only one case of 31 weeks gestational age was there a respiration above 50 per minute during the first 24 hours In this case however there were only slight transient atelectasies on chest X ray and a normal standard bicarbonate was noted Birth weights and lengths were within ± 1 s D for gestational age and sex The 5 full term infants had a gestational age of 38–41 weeks and the 4 early born a gestational age <36 weeks They showed no malformations or infections and the neonatal period was uneventful

1–2 ml of blood were drawn from the cubital or capillary veins into a heparinized tube at 2 4 or 6 hours of age During this period urine was collected in a sterile plastic urine bag The samples were treated as reported elsewhere (15) In 2 of the full term babies an additional blood sample was collected at 7 hours of age 1 hour after the first breastmilk meal The full term infants were taken out of the incubator after this but kept in the same ward (adoption cases)

Samples were collected at 24 and 72 hours of age 3–4 hours after the last breastmilk meal In one of the full term adoption cases where 2 3 cowmilk formula was given from the 4th day of life an additional sample was taken at 15 days of age 4 hours after the last meal In one of the early born babies a sample was drawn at 1 and 3 weeks of age 4 hours after breastmilk and in another at 4 weeks of age 4 hours after 1 3 cowmilk formula

Complete analysis on a Beckman 120 B analyzer was made on pooled samples taken from the same individual at different times during the given periods

## DETERMINATION OF INDIVIDUAL AMINO ACIDS

Eighteen free amino acids plus urea and glutamine were determined on pooled plasma samples according to the method described earlier (15) on a Beckman 120 B amino acid analyzer The estimations of glutamine levels were done after acid hydrolysis with 2 $N$ HCl at 110 C for 2 hours in evacuated sealed tubes (29) Th urine was brought to pH 11

This study was made possible through grants from Expressen s Prenatal Fund AB Semper s Fund for Nutritional Research and Grant No 2583 from the Swedish Medical Research Council

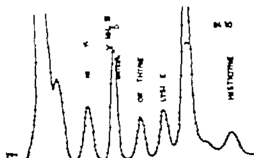



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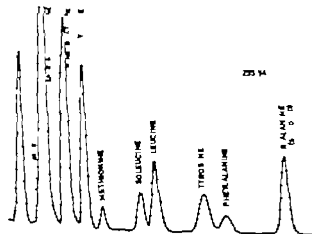



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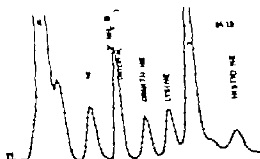


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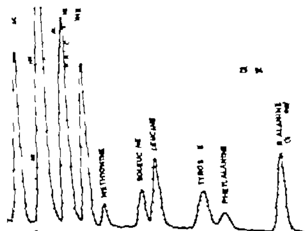


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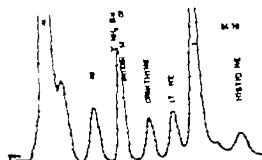



*Fig 1* Chart No 84 10 Chromatogram showing the basic amino acids studied in 300 µl of plasma of an early born baby at one week of age Gamma amino butyric acid was added to the sample as internal standard for colour yield

and lyophilized in order to remove ammonia and then readjusted to pH 2 0-2 2

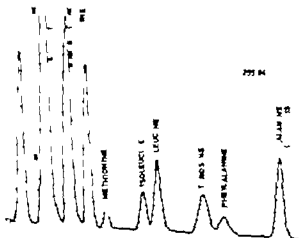
A modified system of ion exchange chromatography was worked out in order to achieve reproducible determinations of essential amino acid levels on small amounts of plasma Tryptophan which is partly bound to plasma albumin (19) and is lost upon deproteinization and deproteinization (29) and threonine which is overlapped by serine and glutamine on chromatography using sodium citrate buffers were excluded in this study Histidine which is an essential amino acid during growth and tyrosine which is essential to the foetus due to the lack of phenylalanine hydroxylase activity in the liver of foetuses (13) were included in the study

The equipment consisted of two Beckman Accu Flo pumps an LKB multichannel absorptiometer Type 5950 B fitted with 3 mm cuvettes and a Philips 1-channel recorder PR 4069 m 04 with a two fold linear amplification The temperature of the column was kept constant by means of a Pappi Colora thermostat K 4000 and the water bath by means of an Hopad 1 omantle at 200 V The composition of the buffers and ninhydrine solution was that described by Spackman *et al* (28)

To 300 µl of plasma were added 1.5 ml of 1.2 % picric acid The precipitation was removed by centrifugation at room temperature and an aliquota of 1.5 ml withdrawn lyophilized and stored at −20°C The dry sample was dissolved in 270 µl of 0 1 − N HCl excess picric acid was removed by centrifugation at room temperature 200 µl of the supernatant plus 25 µl of 1 15 10⁻⁴ − N aminobutyric acid as internal standard were loaded on the column

The basic amino acids were determined as follows The column size was 3×300 mm and Zeolite 225 (NC 8 % spherical particles) was used as resin over a combined teflon + steel filter After each analysis the resin was washed with 1 ml of 0 2 − N NaOH containing 0 67 g of BRIJ 35/liter and then reequilibrated with 1 ml of the pH 4 26 buffer The tube volume between the pump and the column plus the space above the resin was 4 3 ml After loading the sample and filling the space above the resin with pH 4 26 buffer the pH 5 8 buffer was pumped through the system at a flow rate of 8 0 ml/hour The flow rate of the ninhydrine solution was 4 0 ml/hour The temperature was kept at 27 2 C Picric acid emerged as a sharp peak after and overlapping the acidic and neutral amino acids but left the rest of the chromatogram as well resolved peaks (Fig 1) Histidine was eluted after 10 minutes and arginine emerged as a broad flat peak after a further 170 minutes Temperature flow rate and volume of the pH 4 26 buffer were very critical and small alterations gave a poor resolution or a bad reproducibility Contamination of the buffers with ammonia easily gave a plateau which made integration of lysine and histidine peaks difficult The integration was made by the height times width method

The neutral amino acids were determined according to the following modifications The column size was 3x650 mm and Beckman resin AA 15 was used which gave a better resolution of the neutral amino acid than the Zeolite previously used The column was washed with 4 ml of 0 2 − N NaOH + BRIJ 0 67



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# TIME STUDIES ON FREE AMINO ACID LEVELS OF VENOUS PLASMA DURING THE NEONATAL PERIOD

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The aminogram of human cord vein plasma is characterized by hyperaminoacidemia with a marked increase of the lysine and taurine levels (15) The high levels decline towards the normal levels of infancy (27) about a week after birth (11 23) The plasma levels of tyrosine and proline are known to stay high for a longer period (4 11 14)

Studies of the plasma amino acid homeostasis in the neonatal period could give valuable human correlation to the knowledge of foetal and neonatal protein metabolism in animals The need for early screening tests of inborn errors of amino acid metabolism makes such studies necessary

A method of ion exchange chromatography on small amounts of human plasma has therefore been worked out and has been applied in a survey of venous plasma free amino acid levels of the normal human full term and early born during the neonatal period

## EXPERIMENTAL

The material is based on 9 newborns 5 full term and 4 early born They were all taken after an uneventful normal delivery to an incubator in a newborn unit within one hour of delivery They showed temperatures >35 C upon arrival at the incubator There had been no signs of intrauterine asphyxia and the Apgar's score had been 10 at 4 minutes after delivery In only one case of 31 weeks gestational age was there a respiration above 50 per minute during the first 24 hours In this case however there were only slight transient atelectases on chest X ray and a normal standard bicarbonate was noted Birth weights and lengths were within ± 1 S D for gestational age and sex The 5 full term infants had a gestational age of 38–41 weeks and the 4 early born a gestational age <36 weeks They showed no malformations or infections and the neonatal period was uneventful

1–2 ml of blood were drawn from the cubital or capillitual veins into a heparinized tube at 2 4 or 6 hours of age During this period urine was collected in a sterile plastic uri bag The samples were treated as reported elsewhere (15) In 2 of the full term babies an additional blood sample was collected at 7 hours of age 1 hour after the first breastmilk meal The full term infants were taken out of the incubator after this but kept in the same ward (adoption cases)

Samples were collected at 24 and 72 hours of age 3–4 hours after the last breastmilk meal In one of the full term adoption cases where 2 3 cowmilk formula was given from the 4th day of life an additional sample was taken at 15 days of age 4 hours after the last meal In one of the early born babies a sample was drawn at 1 and 3 weeks of age 4 hours after breastmilk and in another at 4 weeks of age 4 hours after 1 5 cowmilk formula

Complete analysis on a Beckman 120 B analyzer was made on pooled samples taken from the same individual at different times during the given periods

## DETERMINATION OF INDIVIDUAL AMINO ACIDS

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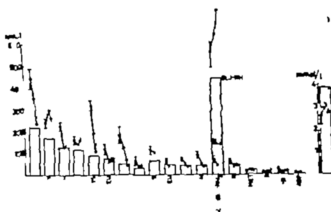


Fig 4 Results of the survey of the plasma levels of 18 free amino acids plus urea and glutamine on pooled plasma samples from full term babies. The order from left to right is according to decreasing levels in adult plasma The columns represent the normal levels of infants (27) — normal cord vein levels ± double standard error (15) O = plasma level after the first neonatal hours of fasting ● = plasma levels at 1 3 days of age × = plasma levels at 15 days of age 4 hours after cowmilk formula feeding

could be due to some losses during the deproteinization in the preparation of plasma for the automatic analyzer a step which was eliminated in the present method Microbiological assay of histidine and lysine (5) on the same samples gave slightly but not significantly higher results than those of the method described here

Reproducibility in the aminogram is demonstrated by 12 peaks representing the internal standard from one curve chromatograms (Fig 3) The reproducibility of the internal standards was better than ± 5 Reproducibility of runs with standard solutions (Bio Rad amino acid calibration mixture) or the precision of the instrument was ± 2% with a load of 50 nmol per amino acid The c values from the standard runs varied during the 7 months of analyses with ± 4

## RESULTS

A The result of the surveys on pooled plasma samples are given in Table 1 During the first hours of fasting there was a considerable general decline in the levels of all amino acids except glycine (Fig 4) Glutamine and urea increased during this period In the early born there was also an increase of the tyrosine and taurine levels The levels of valine (Fig 5) leucine isoleucine and glutamic acid declined already during the first postnatal hours to low levels for normal infants

During the next 3 days of postnatal life with increased intake of breastmilk there was a continuation of the general decline with the exception of tyrosine proline and glutamine levels

After feeding the term infant with a high protein formula (2 3 ) from the 4th to 15th day of life there was a marked increase of the

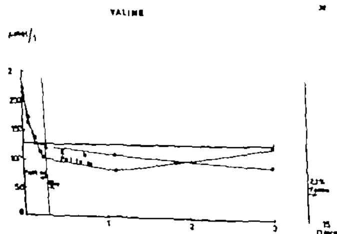



Fig 5 Free valine levels of venous plasma during the neonatal period o = level one hour after first breastmilk feeding in the full term The horizontal line represents the normal level of infants (27)

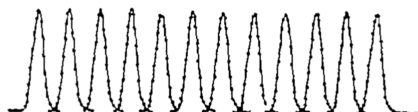


*Fig 3* Peaks representing the interna standard of beta alanine from 12 succes sive chromatograms Reproducibility was better than ± 5 %

g/l and regenerated with 4 ml of a pH 3 43 ± 0 03 buffer After loading the sample the pH 3 43 buffer was pumped through the system at a rate of 17 ml/hour The flow rate of the ninhydrine solution was 8 ml/hour *The total flow rate varied in between 24 5* and 25 2 ml/hour through the time of the actual determinations The temperature was kept at 61 8 C Picric acid emerged very early and showed a well resolved peak overshadowing the taurine peak (Fig 2) 57 mμM of β alanine was used as an internal standard and emerged after 240 minutes Temperature and pH of the buffer were of critical importance to a good separation A higher temperature gave a better separation of tyrosine and phenylalanine but a worse separation of isoleucine and leucine A lower pH of the buffer gave a bad separation of cystine an valine

Reproducibility of the method described was fo isoleucine ± 1 % valine and ornithine ± 3 % tyrosin ± 4 % histidine lysine leucine and phenylalanin ± 5 while methionine showed a reproducibility o ± 10 %. The load on the columns ranged from abo 70 mμmol (lysine) to 8 mμmol (methionine) Th reproducibility was thus with the exception of me thionine whose normal plasma level is very low to b compared with that of the automatic amino ac analyzer (± 5 % (15))

When the same plasma samples were run in the automatic analyzer the results were found to show down to 10 % lower values for all amino acids The

Table 1 *Time study of 18 free amino acid plus glutamine and urea levels on pooled plasma samples from 2 full term and one early born during the neonatal period*

Mean values are given expressed in μmol per l plasma The concentrations in the urine are also given in μmol per l The order from top to bottom is according to decreasing disappearance from plasma during the first hours fasting in the full term By disappearance from plasma is here meant cord vein plasma levels (15) minus cubital vein plasma levels after the first hours fasting The samples taken at 1–3 days were drawn 3–4 hours after the last breastmilk meal The later samples were taken 4 hours after the last meal 2 3 cowmilk formula at 15 days of age in the full term and 1 5 cowmilk formula at 4 weeks of age in the early born

| Amino acid | After first hours fasting | | Disappearance from plasma first hours ( ) | | After 1–3 days | | After cow milk formula | | First hours urine | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Term | Early born | Term | Early born | Term | Early born | Term (2 3 protein) | Early born (1 5 protein) | Term | Early born |
| *Isoleucine* | 30 | 13 | 52 | 40 | 26 | 22 | 101 | 58 | Tr | Tr |
| Leucine | 60 | 55 | 49 | −49 | 62 | 55 | 207 | 100 | Tr | 9 |
| α-NH₂-Bu | 13 | 13 | 46 | 13 | 15 | 10 | 15 | 6 | Tr | Tr |
| Ornithine | 48 | 52 | 46 | 37 | 36 | 46 | 75 | 82 | 31 | 49 |
| Tyrosine | 35 | 66 | 43 | + 2 | 40 | 124 | 154 | 59 | 51 | 18 |
| Valine | 129 | 156 | −42 | −35 | 100 | 83 | 345 | 147 | 36 | 16 |
| Glutamic acid | 40 | 67 | 41 | 27 | | 57 | | 700 | 176 | 83 |
| Lysine | 191 | 230 | 40 | 39 | 106 | 92 | 228 | 148 | 666 | 87 |
| Arginine | 42 | 28 | −35 | 61 | 43 | 70 | 84 | 74 | 31 | Tr |
| Taurine | 121 | 227 | −33 | 21 | 65 | 88 | 39 | 69 | 5030 | 3030 |
| Aspartic acid | 7 | 7 | −30 | 61 | | 9 | | 16 | Tr | Tr |
| Phenylalanine | 49 | 47 | −27 | 35 | 35 | 42 | 64 | 46 | 39 | 14 |
| Methionine | 14 | 23 | −22 | 32 | 13 | 17 | 36 | 30 | Tr | 6 |
| Alanine | 353 | 335 | −20 | −45 | 233 | 158 | 410 | 224 | 358 | 90 |
| Proline | 141 | 139 | −12 | − 6 | 157 | 188 | 312 | 200 | 110 | 71 |
| Histidine | 102 | 77 | − 9 | −39 | 64 | 71 | 102 | 84 | 1170 | 172 |
| Citrulline | 9 | 8 | 00 | −11 | Tr | 7 | 13 | 6 | Tr | Tr |
| Glutamine | 630 | | +15 | | 736 | | | | | |
| Urea | 3100 | 3920 | +20 | +22 | 2750 | 3550 | 6930 | 915 | 83600 | 21500 |
| Glycine | 299 | 229 | +25 | +14 | 264 | 227 | 194 | 230 | 910 | 390 |



*Fig. 4* Results of the survey of the plasma levels of 18 free amino acids plus urea and glutamine on pooled plasma samples from full term babies. The order from left to right is according to decreasing levels in adult plasma. The columns represent the normal levels of infants (27) — normal cord vein levels ± double standard error (15) O = plasma level after the first neonatal hours of fasting ● = plasma levels at 1 3 days of age × = plasma levels at 15 days of age 4 hours after cow milk formula feeding

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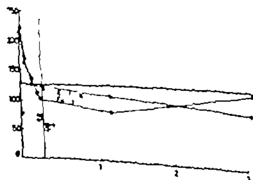



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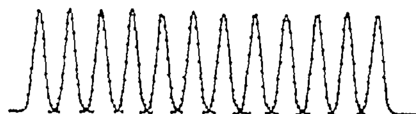


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| | After first hours fasting | | Disappearance from plasma first hours ( ) | | After 1–3 days | | After cow milk formula | | First hours urine | |
|---|---|---|---|---|---|---|---|---|---|---|
| Amino acid | Term | Early born | Term | Early born | Term | Early born | Term (2 3 protein) | Early born (1 5 protein) | Term | Ea ly born |
| Isoleucine | 30 | 33 | 52 | −40 | 26 | 22 | 101 | 58 | Tr | Tr |
| Leucine | 60 | 55 | 49 | 49 | 67 | 55 | 207 | 100 | Tr | 9 |
| *α-NH₂-Bu* | *13* | *13* | −46 | *13* | *15* | *10* | 15 | 6 | Tr | Tr |
| Ornithine | 48 | 52 | −46 | 37 | 36 | 46 | 75 | 82 | 31 | 49 |
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| Lysine | *191* | 230 | −40 | −39 | *106* | 92 | 228 | 148 | 666 | 87 |
| Arginine | 42 | 28 | −35 | −61 | 43 | 20 | 84 | 74 | 31 | Tr |
| Taurine | 121 | 227 | −33 | +21 | 6[illegible] | 88 | 39 | 69 | 5030 | 3030 |
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| Phenylalanine | 49 | 47 | −27 | −35 | 3[illegible] | 42 | 64 | 46 | 39 | 14 |
| Methionine | 14 | 23 | −22 | −32 | 13 | 17 | 36 | 30 | Tr | 6 |
| Alanine | 333 | 235 | −20 | −45 | 233 | 158 | 410 | 224 | 358 | 90 |
| Proline | *141* | *139* | *−12* | *− 6* | *157* | *188* | 317 | 200 | 110 | 71 |
| Histidine | 102 | 77 | 9 | −39 | 84 | 71 | 107 | 84 | 1170 | 172 |
| Citrulline | 9 | 8 | 00 | −11 | Tr | 7 | 13 | 6 | Tr | Tr |
| Glutamine | 630 | | +15 | | 736 | | | | | |
| Urea | 3100 | 3920 | +20 | +22 | 2750 | 3550 | 6930 | 915 | 83600 | 21500 |
| Glycine | 299 | 229 | +25 | +14 | .64 | 227 | 194 | 230 | 930 | 390 |

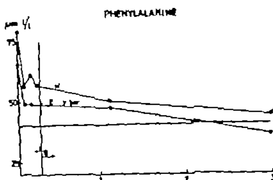



Fig 7 Free phenylalanine levels of venous plasma during the neonatal period

essential amino acids studied by this method with the exception of tyrosine in the early born (Fig 8) Isoleucine leucine valine and histidine fell to low levels compared to normal infants already during the first few hours of fasting (Figs 5 and 6) while the decrease in phenylalanine was less marked (Fig 7)

One hour after the first breastmilk meal at 6 hours of age the plasma levels of leucine isoleucine and valine had increased while those of tyrosine phenylalanine and methionine continued to show a decrease The survey of basic amino acid levels at 1 and 3 weeks of age showed small variations around the normal levels of infants

## DISCUSSION

In the modified system of ion exchange chromatography on small amounts of plasma deproteinization of the sample on a Dowex column was omitted This could mean a greater accuracy in view of reported losses upon deproteinization (29) Another advantage of the method was the fact that no buffer change was needed in the chromatography of the neutral amino acids which simplified the equipment and procedure The cost of the equipment was considerably reduced in comparison to that of a commercial automatic analyzer while the reproducibility was the same

From Table 1 it is evident that the fast decline of the plasma amino acid levels is not accompanied by a corresponding urinary excretion The branch-chained amino acids in particular show a marked decline in plasma during the first hours of extrauterine life (Figs 5 and 6) while they are only represented by trace amounts in the urine (Table 1) Soremi *et al* (24) found a low urinary clearance of

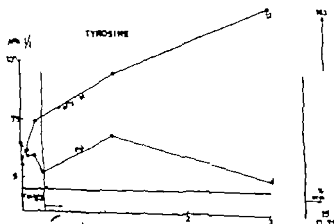



*Fig 8* Free tyrosine levels of venous plasma during the neonatal period.

Table 2 *Time study of essential amino acid plus ornithine levels on individual plasma samples duri the neonatal period*

Mean values are given expressed in μmol per l plasma The 7 hours samples were taken 1 hour after the first brea milk meal the 24 and 72 hours samples at 3–4 hours after last breastmilk meal and the subsequent samples 4 hou after last meal Cow milk feeding is indicated by percentage of protein in the formula used The neutral amino a are based on 3 full term and 3 early born the basic on one full term and one early born

| Amino acid | Term | | | | | | | | Early born | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Neutral | Cord vein | 2 hrs | 4 hrs | 6 hrs | 7 hrs | 24 hrs | 72 hrs | 15 days (2 3 protein) | Cord vein | 2 hrs | 4 hrs | 24 hrs | 72 hrs | 4 wks (1 5 prote |
| Isoleucine | 60 | 37 | 30 | 25 | 32 | 24 | 32 | 104 | 76 | 40 | 33 | 39 | 21 | 58 |
| Leucine | 109 | 71 | 66 | 48 | 65 | 51 | 74 | 214 | 120 | 68 | 57 | 65 | 63 | 100 |
| Valine | 213 | 162 | 137 | 104 | 121 | 82 | 123 | 384 | 221 | 171 | 126 | 109 | 90 | 147 |
| Tyrosine | 65 | 59 | 60 | 52 | 45 | 69 | 50 | 163 | 56 | 61 | 75 | 96 | 123 | 59 |
| Phenyl alanine | 79 | 56 | 61 | 57 | 49 | 50 | 44 | 69 | 66 | 49 | 49 | 47 | 36 | 46 |
| Methionine | 28 | 27 | 27 | 25 | 23 | 22 | 16 | 43 | 26 | 26 | 18 | 26 | 19 | 30 |

| Basic | Cord vein | 6 hrs | 24 hrs | 72 hrs | 1 wk | Cord vein | 24 hrs | 72 hrs | 1 wk | 3 wks | 5 wks (2 3 prote |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Lysine | 328 | 230 | 119 | 139 | 187 | 377 | 113 | 160 | 132 | 69 | 157 |
| Histidine | 110 | 60 | 50 | 66 | 79 | 126 | 26 | 71 | 49 | 52 | 103 |
| Ornithine | 83 | 56 | 37 | 74 | 72 | 82 | 26 | 37 | 52 | 28 | 69 |

levels of all amino acids except glycine and taurine The urea level was also markedly in creased

The amino acid concentration in the urine during the first hours seems to be characterized by high concentrations of taurine histidine glycine and lysine There were lower concen trations of th same amino acids in the early born The taurine excretion was predominan The full term infant also seemed to excret cystine cysteic acid and hydroxyproline dur ing the first neonatal hours

B The results of the time studies on ind vidual plasma samples are given in Table 2 I general there was a rapid fall already durin the first 24 hours of the plasma levels of a

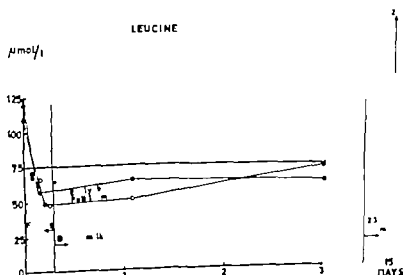



*Fig* 6 Free leucine levels of venou pla ma during the neona al period

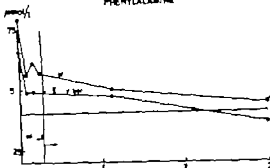






*Fig 7* Free phenylalanine levels of venous plasma during the neonatal period

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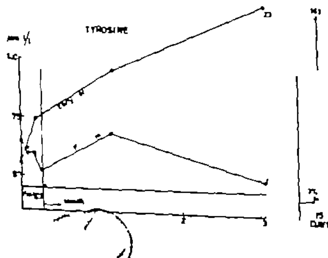



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| Amino acid | Term | | | | | | | | Early born | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Neutral | Cord vein | 2 hrs | 4 hrs | 6 hrs | 7 hrs | 24 hrs | 72 hrs | 15 days (2 3 protein) | Cord vein | 2 hrs | 4 hrs | 24 hrs | 72 hrs | 4 wks (1.5 protein) |
| Isoleucine | 60 | 37 | 30 | 25 | 32 | 24 | 32 | 104 | 76 | 40 | 33 | 39 | 21 | [illegible] |
| Leucine | 109 | 71 | 66 | 48 | 65 | 51 | 74 | 214 | 120 | 68 | 57 | 65 | 63 | 100 |
| Valine | 213 | 162 | 137 | 104 | 121 | 82 | 123 | 384 | 221 | 171 | 126 | 109 | 90 | 147 |
| Tyrosine | 65 | 59 | 60 | 52 | 45 | 69 | 50 | 163 | 56 | 61 | 75 | 96 | 123 | [illegible] |
| Phenyl alanine | 79 | 56 | 61 | 57 | 49 | 50 | 44 | 69 | 66 | 49 | 49 | 47 | 36 | 46 |
| Methionine | 28 | 27 | 27 | 25 | 23 | 22 | 16 | 43 | 26 | 26 | 18 | 26 | 19 | 10 |

| Basic | Cord vein | 6 hrs | 24 hrs | 72 hrs | 1 wk | Cord vein | 24 hrs | 72 hrs | 1 wk | 3 wks | 5 wks (2 3 protein) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Lysine | 328 | 230 | 119 | 139 | 187 | 377 | 113 | 160 | 132 | 69 | 157 |
| Histidine | 110 | 60 | 50 | 66 | 79 | 126 | 56 | 71 | 49 | 57 | 103 |
| Ornithine | 83 | 56 | 37 | 74 | 72 | 82 | 26 | 37 | 57 | 28 | [illegible] |

levels of all amino acids except glycine and taurine The urea level was also markedly increased

The amino acid concentration in the urine during the first hours seems to be characterized by high concentrations of taurine histidine glycine and lysine There were lower concentrations of the same amino acids in the early born The taurine excretion was predominant The full term infant also seemed to excret cystine cysteic acid and hydroxyproline during the first neonatal hours

B The results of the time studies on individual plasma samples are given in Table 2 In general there was a rapid fall already during the first 24 hours of the plasma levels of all

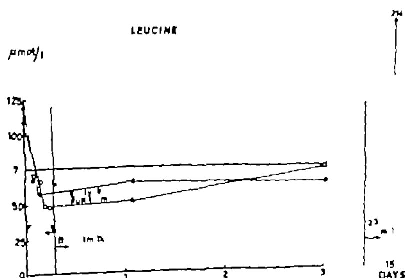



*Fig 6* Free leucine levels of venous plasma during the neonatal period

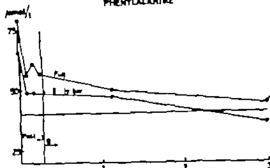





Fig 7 Free phenylalanine levels of venous plasma during the neonatal period

essential amino acids studied by this method with the exception of tyrosine in the early born (Fig 8) Isoleucine leucine valine and histidine fell to low levels compared to normal infants already during the first few hours of fasting (Figs 5 and 6) while the decrease in phenylalanine was less marked (Fig 7)

One hour after the first breastmilk meal at 6 hours of age the plasma levels of leucine isoleucine and valine had increased while those of tyrosine phenylalanine and methionine continued to show a decrease The survey of basic amino acid levels at 1 and 3 weeks of age showed small variations around the normal levels of infants

## DISCUSSION

In the modified system of ion-exchange chromatography on small amounts of plasma deproteinization of the sample on a Dowex column was omitted This could mean a greater accuracy in view of reported losses upon deproteinization (29) Another advantage of the method was the fact that no buffer change was needed in the chromatography of the neutral amino acids which simplified the equipment and procedure The cost of the equipment was considerably reduced in comparison to that of a commercial automatic analyzer while the reproducibility was the same

From Table 1 it is evident that the fast decline of the plasma amino acid levels is not accompanied by a corresponding urinary excretion The branch-chained amino acids in particular show a marked decline in plasma during the first hours of extrauterine life (Figs 5 and 6) while they are only represented by trace amounts in the urine (Table 1) Sereni *et al* (24) found a low urinary clearance of

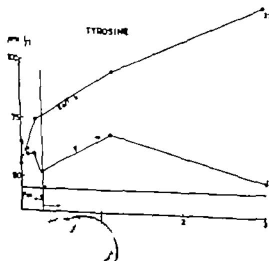





Fig 8 Free tyrosine levels of venous plasma during the neonatal period

these amino acids during the neonatal period There is evidence in favour of an increased hepatic transfer of amino acids in the immediate neonatal period (8) However, perfusion of isolated liver shows 90 uptake of free amino acids while the uptake of branch chained amino acids is low (17) Thus it seems as if the changes seen could be due to an intensive extrahepatic as well as hepatic transfer The lowering of the branch-chained amino acid plasma levels after hepatectomy in the dog (18) speaks in favour of their ready utilization in the periphery The transhepatic shunt of the foetus in combination with the high levels of growth hormone in cord vein blood (30) would favour extrahepatic transfer of amino acids to brain gut etc After transamination the branch-chained amino acids are metabolized *via* propionyl CoA and acetyl CoA and could be a source of energy during this period The increase in glutamine and urea (Fig 4) and the increased ammonia partition in newborn infants urine (2) is in agreement with the assumption of increased transamination during this period

The 60 fall of arginine and aspartic acid during the first hours fasting (Table 1) while the urea level in plasma increases is in agreement with the finding of considerable arginine synthetase activity during early gestation of the human foetus (21)

The accumulation of tyrosine in the plasma of the early born (Fig 8) is in agreement with the known high levels of tyrosine later in the neonatal period (14 4) and the lack of enzyme activity of the oxidation of tyrosine in the human foetus (13) Lack of phenylalanine hydroxylase activity in the foetal liver has been demonstrated (13) and decreased tolerance of phenylalanine in the normal newborn has been reported (6) In this survey the phenylalanine levels showed a slow decline to that of the normal infant during the first days increase in breastmilk consumption (Fig 7) The increase of the proline level has also been reported by Ghadimi *et al* (11) in combination with the increase of tyrosine after day 2 This finding may depend on the relatively high level of proline in breastmilk (22) and the low clearance of proline in the kidney of premature infants (24)

A high urinary excretion of α amino nitrogen in infants has been a well known fact for over 50 years (25) The high concentrations of histidine glycine and lysine in the first hours urine is in accordance with the especially high urinary clearance of these amino acids found in the neonatal period (24)

The homeostasis of taurine in the foetus and newborn seems to be unique The free taurine level of human foetal liver is increased (20) The ratio of foetal/maternal plasma levels of taurine is very high over 4/1 (15) The plasma levels of taurine fall rapidly to normal during the first days of extrauterine life except in the early born, where there was a temporary increase The high excretion of taurine in the newborn (Table 1) is well known and was first described as a transient characteristic of the normal full term neonate s urine by Bickel & Souchon (3) The taurine level of the rabbit brain is known to decrease during the neonatal period (1) These facts together may suggest that there is an efficient excretion of foetally accumulated taurine during the neonatal period One aspect of this efficient excretion is the inverted ratio of glycocholic acid/taurocholic acid found in the newborn (0 47 against 0 95 at one week and 3 1 in the adult) (9)

The synthesis of taurine has mostly been studied in adult animals The tissues of chick and calf embryos exhibit certain peculiarities of sulphur metabolism which distinguish them from the tissues of adult animals Thus 65 of ³⁵S sulphate administered to the chick embryo at 24 hours of age is recovered as taurine at one day of postnatal age (16) It has also been shown that cysteate in the calf embryo is rapidly decarboxylated to taurine (7) It thus seems that taurine in the chick and calf embryo could be an end product in the catabolism of sulphur-containing amino acids By contrast the adult animal excretes 75 of sulphur in the form of sulphate

The paradoxal behaviour of glycine with high plasma levels on low prot in intake and low levels upon increased protein intake (Fig 4) has also been described in infants by Snyderman *et al* (26) who propos d that the high level reflect subclinical undernutrition of non essential amino acids The high urea level upon cowmilk formula feeding during the neonatal period has been reported earlier (12)

The post prandial increase of branch chained amino acids in the plasma of newborn is in agreement with observations in adults (10) The increase of the leucine level was most marked The first meal might be regarded as a leucine load since the plasma levels of leucine after the first hours fasting are low and the content of leucine in breastmilk is high (still higher in cowmilk formula)

## SUMMARY

A simplified method of ion exchange chromatography of essential amino acids on small amounts of human plasma is described The precision of the instrument and the reproducibility was to be compared with those achieved with automatic amino acid analyzers The method has been applied in a survey of venous plasma free amino acid levels and urinary concentration during the neonatal period

A considerable general decrease of the free amino acid levels was seen already during the first postnatal day This was most marked in the branch-chained amino acids where levels that were low for infants were seen already after the first hours of starvation The well known high urinary excretion of amino acid nitrogen was found to be due to a high excretion of a limited numb r of amino acids

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*Key words* Amino acids plasma newborns ion-exchange chromatography

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# MEASLES VACCINATION

## VII Follow up Studies in Children Immunized with Four Doses of Inactivated Vaccine

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The present studies were undertaken to evaluate the immunizing capacity of purified hemagglutinin prepared from Tween 80 and ether treated material (2) This product—the Tween ether (TE) vaccine—and for comparison formalin killed (FK) whole virus vaccine were employed in two small scale field trials The effectiveness of the two vaccines both with regard to their capacity to induce antibodies when used for primary immunization and to elicit anamnestic responses were analysed (5–10) Children immunized with 3 monthly doses of inactivated vaccine were found to respond with high titers of neutralizing and HI antibodies These antibody titers decreased to low or moderate levels within 6 to 12 months Administration of a booster injection markedly improved the state of immunity However some vaccinees who had received 4 doses of vaccine contracted a subclinical infection when exposed to cases of regular measles This infection occurred in the presence of clearly demonstrable titers of circulating antibody which implies that antibodies induced by inactivated vaccines have a markedly lower protective value than antibodies present in gammaglobulin Furthermore exposure to measles in the presence of significant amounts of circulating antibody induced by immunization with FK vaccine led in two cases to the appearance of atypical complications in the form of pneumonia The present paper describes results of additional serological and clinical follow up studies during a period of 3 to 4 years after a booster injection

## MATERIAL AND METHODS

*Study population* Table 1 gives a summary of the performance of the _ field trials which will be discussed in the following

In trial no I children aged 1/2 to _ years were given a primary immunization with 3 monthly doses of FK vaccine (Chas Pfizer & Co) (5 6) and 22 to 25 months later a booster injection either with the same product or with TE vaccine of high potency (8) Results of a follow up study 18 months after this boostering have already been described (10) Further follow up examinations were made after an additional interval of 29 months The vaccinees were questioned about exposure to measles and finger tip blood samples were collected

Children from field trial no II also received their primary immunization at the age of 1/2 to 2 years but were given three monthly doses either of FK vaccine or of TE vaccine of high potency (7) A booster with TE vaccine of moderate potency was then given to all children 17 months later (9) These vaccinees were interviewed and blood samples were collected on 2 occasions 1 / and 3 years after boostering

*Serological analysis of blood samples* 0 3 ml finger tip blood was collected in 1 2 ml of tissue culture medium containing heparin 1 50 000 The erythrocytes were removed by low-speed centrifugation The supernate which was considered to represent a serum dilution of 1 10 was tested in HI tests according to the technique previously described (5 6) Titer values given refer to final dilutions after addition of antigen All sera belonging to the same study group and obtained from bleedings on one occasion were tested simultaneously and parallel tests were performed with sera from the nearest previous bleeding of the same individuals A human measles convalescent serum was used for standardization of the HA antigen and was also included in the tests as a reference to allow comparison with previous titrations

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*Key words* Amino acids plasma newborns ion-exchange chromatography

Table 2 *Decline in HI antibody titers after boostering of children belonging to field trial I*

| | Time after booster injection | | | | |
|---|---|---|---|---|---|
| | 2 weeks | 10 weeks | 8 months | 1½ year | 4 years |
| *TE vaccine*[a] | | | | | |
| No of children | 14 | 8 | 14 | 10 | 5 |
| Geometric mean HI titer[b] | 18 000 | 7 700 | 7 800 | 3 800 | 2,900 |
| Accumulated relative decrease in titer[c] | — | 2.4 | 2.3 | 4 8 | 11 c |
| *FK vaccine*[a] | | | | | |
| No of children | 14 | 11 | 12 | 6 | 5 |
| Geometric mean HI titer[b] | 3 100 | 520 | 600 | 640 | 320 |
| Accumulated relative decrease in titer | — | 5.5 | 4 5 | 6 3 | 12 6 |

[a] Product used for booster injections
[b] Children displaying changes in titers after exposure to measles have been excluded
[c] Only paired sera from two consecutive bleedings were compared

primary immunization were 21 and 20 The reduction in average serum titer of children not exposed to measles of the former group was 3 4-fold in the period from two weeks to 1½ year after boostering A further 2 4 fold reduction in titers occurred during an additional period of 1½ year (Table 3 Fig 2 and 3) giving totally a 8 1 fold reduction Children who had received 4 doses of TE vaccine displayed a more rapid early post booster decline in HI titers During the period from two weeks to 1½ year post booster the average titer decreased 13 1 times in children not exposed to measles A further 2 8 fold decrease in titers (total reduction 36 7 fold) was recorded during another 1½ year (Table 3 Figs 2 and 3)

Table 3 *Decline in HI antibody titers after boostering of children belonging to field trial II*

| | Time after booster injection | | |
|---|---|---|---|
| | 2 weeks | 1½ year | 3 years |
| *TE vaccine*[a] | | | |
| No of children | 27 | 21 | 19 |
| Geometric mean HI titer[b] | 1200 | 230 | 100 |
| Relative decrease in titer[c] | — | 13 1 | 36 7 |
| *FK vaccine*[a] | | | |
| No of children | 23 | 21 | 15 |
| Geometric mean HI titer[b] | 5600 | 2400 | 7000 |
| Relative decrease in titer | — | 3 4 | 8 1 |

[a] Product used for primary immunization
[b,c] See footnotes of Table 2

*Clinical and serological effects of exposure to measles during a three year post booster period* Table 4 gives a summary of reactions towards exposure to cases of regular measles Among children who had received FK vaccine for their primary immunization two exposures were recorded Both children reacted serologically in spite of good pre-exposure HI ti

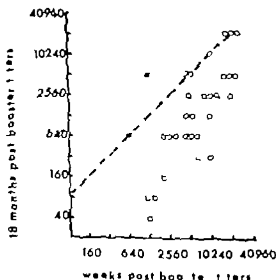



Fig 2 Correlation between serum HI titers two weeks and 18 months after a booster injection to children of field trial no II Different symbols denote children given a primary immunization with FK (o) or TE (□) vaccine Filled symbols refer to children exposed to measles during the time period between the two bleedings

Table 1 *Schedule for field trials with inactivated measles vaccines*

| | Field trial | |
|---|---|---|
| | I | II |
| Primary immunization (3 monthly doses) | FK vaccine | FK vaccine or highly potent TE vaccine |
| Booster injection | | |
| Type of vaccine | FK vaccine or highly potent TE vaccine | Moderately potent TE vaccine |
| Time after primary immunization | 22 to 23 months | 17 months |
| Time for clinical and serological follow ups | | |
| After primary immunization | 8 to 9 months | 11 months |
| After boostering | 2 weeks<br>10 weeks<br>8 months<br>1½ year<br>4 years | 2 weeks<br>1½ year<br>3 years |

## RESULTS

### Field trial I

*Correlation of HI antibody titers 1 ½ and 4 years after a booster injection Effect of clear cut exposure to measles* Nine out of 11 and 5 out of 11 children boostered with FK and TE vaccine respectively and examined 1 ½ year later were available after another 2 ½ years One exposure of a previously unexposed child boostered with FK vaccine had occurred In spite of a HI serum titer of 320 in the 18 months post booster sample this child developed a mild case of measles 2 ½ years after the booster injection There was a fever of 39-40 C for 4 days and a rash mainly located to the feet The HI serum titer 1 ½ year after this infection was 5120

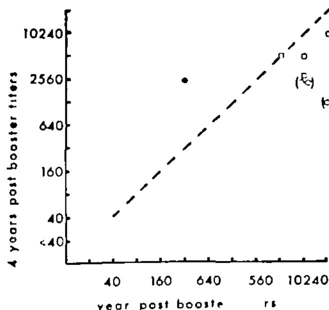



*Fig 1* Correlation between HI serum titers in sera collected 1 ½ and 4 years after a booster injection of either FK (O) or TE vaccine (□) to children belonging to field trial no I The filled circle denotes a child exposed to measles during the time between collection of blood samples and symbols within parentheses refer to children who previously had reacted serologically to an exposure to measles

There was a high degree of correlation between HI serum titers in the 1 ½ and 4 years samples (Fig 1) The average reduction in titers was 2 0 fold in children boostered with FK vaccine and previously not exposed to measles and 2 3 fold in non exposed children who had received TE vaccine (Table 2) Thus the latter titers remained on a level corresponding to that of early measles convalescent sera

### Field trial II

*Correlation of HI antibody titers in blood samples collected 2 weeks and 1 ½ and 3 years after a booster injection* Among 23 children who had received a primary immunization with FK vaccine 21 and 16 vaccinees were available for follow up studies 1 ½ and 3 years after the booster injection respectively The corresponding figures for the 27 children who had instead received TE vaccine for their

Table 2 *Decline in HI antibody titers after boostering of children belonging to field trial I*

| | Time after booster injection | | | | |
|---|---|---|---|---|---|
| | 2 weeks | 10 weeks | 8 months | 1½ year | 4 years |
| *TE vaccine*[a] | | | | | |
| No of children | 14 | 8 | 14 | 10 | 5 |
| Geometric mean HI titer[b] | 18 000 | 7 700 | 7 800 | 3 800 | 2 900 |
| Accumulated relative decrease in titer[c] | — | 2 4 | 2 3 | 4 8 | 11 0 |
| *FK vaccine* | | | | | |
| No of children | 14 | 11 | 12 | 6 | 5 |
| Geometric mean HI titer[b] | 3 100 | 570 | 600 | 640 | 330 |
| Accumulated relative decrease in titer[c] | — | 5 5 | 4 5 | 6 3 | 12.6 |

[a] Product used for booster injections

[b] Children displaying changes in titers after exposure to measles have been excluded

[c] Only paired sera from two consecutive bleedings were compared

primary immunization were 21 and 20 The reduction in average serum titer of children not exposed to measles of the former group was 3 4 fold in the period from two weeks to 1 1/2 year after boostering A further 2 4 fold reduction in titers occurred during an additional period of 1 1/2 year (Table 3 Figs 2 and 3) giving totally a 8 1 fold reduction Children who had received 4 doses of TE vaccine displayed a more rapid early post booster decline in HI titers During the period from two weeks to 1 1/2 year post booster the average titer decreased 13 1 times in children not exposed to measles A further 2 8 fold decrease in titers (total reduction 36 7 fold) was recorded during another 1 1/2 year (Table 3 Figs 2 and 3)

Table 3 *Decline in HI antibody titers after boostering of children belonging to field trial II*

| | Time after booster injection | | |
|---|---|---|---|
| | 2 weeks | 1½ year | 3 years |
| *TE vaccine*[a] | | | |
| No of children | 27 | 21 | 19 |
| Geometric mean HI titer[b] | [illegible] | 230 | 100 |
| Relative decrease in titer | — | 13 1 | 36 7 |
| *FK vaccine*[a] | | | |
| No of children | 23 | 21 | 15 |
| Geometric mean HI titer[b] | 5600 | 2400 | 2000 |
| Relative decrease in titer | — | 3 4 | 8 1 |

[a] Product used for primary immunization

[b] See footnotes of Table 2

*Clinical and serological effects of exposure to measles during a three year post booster period* Table 4 gives a summary of reactions towards exposure to cases of regular measles Among children who had received FK vaccine for their primary immunization two exposures were recorded Both children reacted serologically in spite of good pre-exposure HI ti

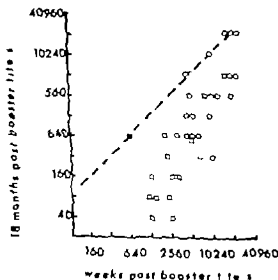



*Fig 2* Correlation between serum HI titers two weeks and 18 months after a booster injection to children of field trial no II Different symbols denote children given a primary immunization with FK ( ) or TE (□) vaccine Filled symbols refer to children exposed to measles during the time period between the two bleedings

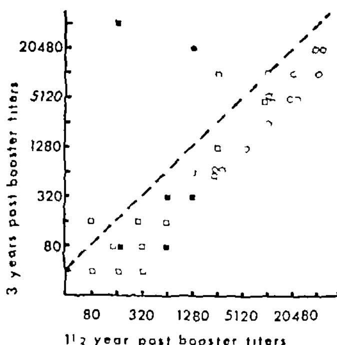



*Fig 3* Correlation between serum HI titers 1½ and 3 years after a booster injection to children of field trial no II For explanation of symbols see legend to Fig 2

ters and one displayed clinical symptoms of a relatively mild character In the group of children who had received 4 doses of TE vaccine 7 cases of presumed exposures had occurred Among these were encountered one case with regular measles, one case with mild symptoms and one case in which the only sign of an infection was a rise in antibody titers One of the vaccinees who did not react had been exposed simultaneously with his older brother This boy had been incompletely immunized 2½ years earlier with two doses of FK vaccine and was therefore not included in the present study He contracted an aberrant form of measles which ran the following course

*Case L E* Ten year old boy taken ill with malaise vomiting and dizziness Two days later respiratory complications in the form of coughing supervened The temperature rose to 40 C Treatment with penicillin was initiated but there was no change in the condition of the patient A week after the appearance of the first symptoms the patient was taken into a hospital for infectious diseases X ray examination revealed the presence of extended partly confluent pneumonic infiltrations in the lower lobe on the left side (Fig 4) After 3 days of hospitalization the patient recovered HI serum antibody titers were 10 about 2 months before the exposure and 64 000 in a blood sample collected during the visit to the hospital

## DISCUSSION

The results presented above of clinical and serological follow up of children immunized with 4 doses of inactivated vaccine give further emphasis to the previously summarized experiences (3) The immunization schedule employed gave high and relatively stable antibody titers Some variations in the titer levels occurred dependent upon whether adjuvant containing (FK) or aqueous (TE) vaccine was used for primary immunization It was noticed before that the degree of sensitization of children who had received TE vaccine was less than that of children immunized with FK vaccine This difference also concerned the stability of titers with time after boostering with TE vaccine of children of these groups The reduction

Table 4 *Clinical and serological findings in children of field trial II reacting to measles exposure after boostering*

| Child no and type of vaccine | Time of exposure in months after boostering | Clinical reactions | Change in HI serum titers in relationship to exposure | |
|---|---|---|---|---|
| | | | Before | After |
| 9 TE | 7 | Mild Low grade fever for 4 days Faint rash | 2560 | 2 560 |
| 79 TE | Unknown | None | 1280 | 5 120 |
| 118 TE | 40 | Regular measles | 160 | 40 960 |
| 17 FK | Unknown | None | 2560 | 10 240 |
| 70 FK | 38 | Mild measles of 2 days duration Low grade fever Faint rash Slight conjunctivitis | 1280 | 20 480 |

Refers to nearest previous and subsequent bleedings



*Fig. 4.* Pulmonary changes in a child (L.L.) contracting an aberrant form of measles 2 1/2 years after immunization with 2 doses of FK vaccine.

of geometric mean HI antibody titers 1 1/2 year after boostering was 13.3 fold and 3.4 fold in children who had received a primary immunization with TE and FK vaccine respectively.

Most vaccinees who had received 4 doses of vaccine were found to be protected when they were exposed to cases of natural measles. However some cases of subclinical or mild measles were encountered. These reactions occurred in spite of the presence of low to moderate titers of antibodies. Since the amount of gammaglobulin needed to confer complete protection theoretically should give barely demonstrable HI serum titers, these antibodies seem to be qualitatively superior to those induced by a killed vaccine. Possibly antibodies which are not picked up by the conventional serological techniques, i.e. the neutralization and HI tests, may be of importance in conferring protection. This problem should be subjected to further studies. An additional complication with the state of immunity induced by killed vaccine is the aberrant forms of measles occasionally encountered in individuals exposed to "wild" virus (1). It seems likely that these pneumonic reactions are due to a unique state of hypersensitization of children who have received inactivated vaccine of the FK type. As yet no pneumonic form of measles has been seen in children immunized with TE vaccine whereas 3 cases of this kind have been encountered among children who have received FK vaccine. However, this difference does not bear significance due to the small number of children included in the field trials. Further studies on the pathogenesis of the aberrant form of measles are warranted.

In the present situation inactivated measles vaccines can not be recommended for general

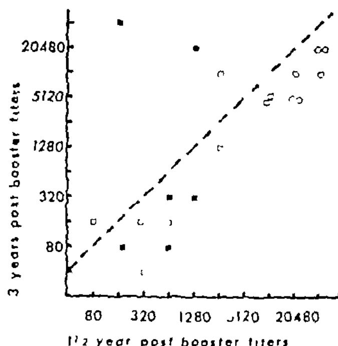



Fig 3 Correlation between serum HI titers 1½ and 3 years after a booster injection to children of field trial no II For explanation of symbols see legend to Fig 2

ters and one displayed clinical symptoms of a relatively mild character In the group of children who had received 4 doses of TE vaccine 7 cases of presumed exposures had occurred Among these were encountered one case with regular measles one case with mild symptoms and one case in which the only sign of an infection was a rise in antibody titers One of the vaccinees who did not react had been exposed simultaneously with his older brother This boy had been incompletely immunized 2½ years earlier with two doses of FK vaccine and was therefore not included in the present study contracted an aberrant form of measles wl ran the following course

*Case 1* F Ten year old boy taken ill with mal vomiting and dizziness Two days later respira complications in the form of con lung superve The temperature rose to 40 C Treatment with p cillin was initiated but there was no chan e in condition of the patient A week after the appear of the first symptoms the patient was taken int hospital for infectious diseases X ray examin revealed the presence of extended partly confl pneumonic infiltrations in the lower lobe on the side (Fig 4) After 3 days of hospitalization the tient recovered HI serum antibody titers were about 2 months before the exposure and 64 000 blood sample collected during the visit to the ho tal

## DISCUSSION

The results presented above of clinical a serological follow-up of children immuniz with 4 doses of inactivated vaccine gave furt emphasis to the previously summarized expe ences (3) The immunization schedule e ployed gave high and relatively stable antibo titers Some variations in the titer levels o curred dependent upon whether adjuvant co taining (FK) or aqueous (TE) vaccine was u for primary immunization It was noticed b fore that the degree of sensitization of child who had received TE vaccine was less th that of children immunized with FK accu This difference also concerned the stability titers with time after boostering with TE va cine of children of these groups The reducti

Table 4 *Clinical and serological findings in children of field trial II reacting to measles exposure after boostering*

| Child no and type of vaccine | Time of exposure in months after boostering | Clinical reactions | Change in HI serum titers in relationship to exposure[a] Before | After |
|---|---|---|---|---|
| 9 TE | 7 | Mild Low grade fever for 4 days Faint rash | 2560 | 2 560 |
| 79 TE | Unknown | None | 1280 | 5 120 |
| 118 TE | 40 | Regular measles | 160 | 40 960 |
| 17 FK | Unknown | None | 2560 | 10 240 |
| 70 FK | 38 | Mild measles of 2 days duration Low grade fever Faint rash Slight conjunctivitis | 1280 | 20 480 |

[a] Refers to nearest previous and subsequent bleedings



*Fig 4* Pulmonary changes in a child (L.E.) contracting an aberrant form of measles 2 / years after immunization with 2 doses of FK vaccine

of geometric mean HI antibody titers 1 1/ year after boostering was 13 3 fold and 3 4 fold in children who had received a primary immunization with TE and FK vaccine respectively

Most vaccinees who had received 4 doses of vaccine were found to be protected when they were expo ed to ca es of natural measles However some cases of subclinical or mild measles were encountered These reactions occurred in spite of the presence of low to-moderate titers of antibodies Since the amount of gammaglobulin needed to confer complete protection theoretically should give barely demonstrable HI serum titers these antibodies seem to be qualitatively superior to those induced by a killed vaccine Possibly antibodies which are not picked up by the conventional serological techniques i e the neutralization and HI tests may be of importance in conferring protection This problem should be subjected to further studies An additional complication with the state of immunity induced by killed vaccine is the aberrant forms of measles occasionally encountered in individuals exposed to "wild" virus (1) It seems likely that these pneumonic reactions are due to a unique state of hypersensitization of children who have received inactivated vaccine of the FK type As yet no pneumonic form of measles has been seen in children immunized with TE vaccine whereas 3 cases of this kind have been encountered among children who have received FK vaccine However this difference does not bear significance due to the small number of children included in the field trials Further studies on the pathogenesis of the aberrant form of measles are warranted

In the present situation inactivated measles vaccin s can not be recommended for general

use Although they offer a number of theoretical advantages, practical experiences gained during the last 5 years leave a number of unsolved problems (3, 4) One difficulty concerns the development of techniques for isolation of envelope antigens to be included in a purified product Other problems concern the state of immunity which can be induced As mentioned the use of killed measles vaccine induces antibodies of relatively low protective value Furthermore, the absence of a local production of IgA antibodies in the respiratory tract may be a disadvantage The absolute need for this kind of antibody cannot be clearly evaluated On one hand it is known that gammaglobulin which contains no or only small amounts of IgA can give a complete protection Furthermore it seems likely that parenterally administered live vaccine should give a rather poor stimulus to IgA production in the respiratory tract, since the vaccine infection displays no tendency to be contagious On the other hand immunization with killed vaccine has been found to confer a complete protection, including prevention of local virus replication only when very high titers of circulating antibodies have been obtained It seems important to reach a high antibody level since the basis for the aberrant pneumonic reaction seen in some vaccinees, may be due to a local antigen antibody reaction It might therefore be worth while making attempts to stimulate preferentially a production of IgA antibodies in the respiratory secretions by a local application of inactivated measles vaccine Immunization with inactivated influenza vaccine has given encouraging results in recent studies (11)

## SUMMARY

Children immunized with 4 doses of formalin killed (FK) whole virus vaccine containing adjuvant and aqueous purified hemagglutinin prepared from Tween ether (TE) treated material in different combinations have been followed serologically and clinically during a period of 3 to 4 years after the last dose of vaccine The average decline in HI antibody titers was about 10 fold during this period of time, except in children who had received [illegible] doses of TE vaccine The latter group displayed about a 40 fold decrease in titers Four out of 10 children exposed to cases of regular measles displayed clinical symptoms of varying degrees of severity Two more children responded with a rise in HI antibody titer only All these reactions occurred in children with a pre exposure HI serum titer of 160 or higher A case of pneumonia was encountered in connection with exposure to wild virus in a child who had received only 2 doses of FK vaccine

## ACKNOWLEDGEMENT

The excellent technical assistance of Miss Ylva Gollmar is gratefully acknowledged

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*Key words* Measles vaccine inactivated immunity

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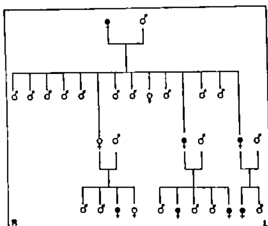


*Fig 1* Pedigree (see text)

The sacral bone anomaly with anterior sacral meningocele in most cases is an oval defect in one side of the distal one third of the sacrum covered by fibrous connective tissue. Moreover there is a deviation of the opposite aspect of the sacrum forming an osseous delineation of hook or sickle shape suggesting that the sacrum has developed around the meningocele. The deformation varies according to the size of the meningocele.

According to Smith (32) congenital sacral disease may be divided into total and subtotal agenesis with one or more segments missing in total hemisacrum where one side is entirely missing and in subtotal hemisacrum where a few segments are absent. The sacral defect with anterior sacral meningocele belongs in the last group.

The anterior meningocele consists of dura mater and arachnoidea often supported by fibrous connective tissue. The cystic wall may contain glial tissue. The meningocele generally herniates through a ventral defect in the sacrum but may also arise from an enlarged foramen intervertebrale or through foramen ischiadicum and appears as a tumor in the gluteal region.

## CASE REPORTS

The following two patients with partial absence of the sacrum and the coccyx associated with sacral ventral meningocele were admitted to the Department of Pediatrics of Glostrup Hospital. Both were girls aged 3 and 8 respectively. They belonged to the same family (Fig 1). The same congenital malformation was also present in other female family members suggesting a sex linked dominant inheritance which has not been described in previous studies of this complex of malformations.

To the two cases mentioned above we have added four from the family.

*Case no 1*

A three year old girl (210165 W h) cousin of patient no born as no 5 of 5 siblings. No 1 a boy died suddenly four months old on unknown cause no 2 a girl was clinically healthy but sacral and coccygeal absence had been disclosed by radio-



*Fig* Radiogram showing the typical hook shaped sacral defect (case no 1)

*Acta Pædiat Scand 58* 268–274 1969

# HEREDITARY DEFECT OF THE SACRUM AND COCCYX WITH ANTERIOR SACRAL MENINGOCELE

JORGEN COHN and ERIK BAY NIELSEN

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The anterior sacral meningocele arises from a partial or total defect of the sacrum and the coccyx, through which the caudal part of meninges herniates like a cyst

Partial or complete absence of the sacral and the coccygeal bones was first described by Hohl in 1852 and in 1857 Wertheim published a case of complete sacro-coccygeal agenesis In the period from 1857 to 1959 a total of 50 cases have been published In 1959 Blumel *et al* (4) described 50 cases including 32 with complete agenesis Of the 50 patients 23 were women A great number of the patients had other malformations Thus 14 had spina bifida 5 meningocele 27 pes equinovarus 4 paralysis of the limbs and 5 atrophy of the limbs 10 had dysfunction of the bladder or intestine 5 had anal defects Six of the patients had been born by diabetic mothers Other workers have found associated malformations such as congenital anal stenosis ureteral reflux with hydronephrosis and uterus didelphys (4 13 29)

Since 1959 further cases have been published so that in 1966 a total of 111 cases were on record, including 69 of anterior sacral meningocele

Coller & Jackson in 1943 (9) published 23 cases of anterior sacral meningocele with sacral defect Of these 20 were women Until 1950 34 cases had been published, of which 26 were women Out of 34 patients 10 had associated congenital anomalies Leigh *et al* (24) in 1954 described 4 cases, all of which were female Of the 69 cases reported until 1966 sixty were female The sex predominance may be ascribed to the fact that meningocele is frequently diagnosed in women in connexion with delivery complications

## ETIOLOGY AND PATHOGENESIS

The sacrum in early fetal life consists of five isolated segments which unite at a time varying from shortly after birth to the age of 30

Anterior sacral meningocele must be considered a pathological deviation of a phylogenetical process The origin of spinal malformations and thus of the myelocele and meningocele according to Gardner (15) and Cramer (10) is changes in the neural tube in embryonic life with dilatation of the neuenteric canal According to Gardner and Cramer this dilatation is due to reduced permeability of the roof of the fourth ventricle of the brain which interferes with the passage of liquor into the subarachnoid space Consequently the liquor pressure increases and the normal collapse of the neuenteric canal fails to occur This may lead to defective closure of the neural tube posteriorly and anteriorly and myelo meningoceles or meningoceles may develop anywhere in the spinal canal At the same time defective fusion of the sclerotomas may cause bone defects both anteriorly and posteriorly throughout the vertebral column

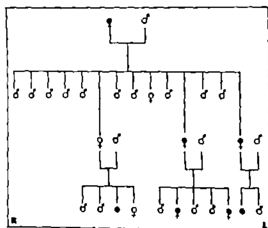


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Fig 2 Radiogram showing the typical hook shaped sacral defect (case no 1)



*Fig 3* Lateral view of the lumbo sacral region The Pantopaque material is visible in the spinal cord and in the meningocele anteriorly to the sacrum (case no 1)

graphy (Fig 2) No 3 a boy had congenital anal atresia He died 8 days old of postoperative peritonitis No 4 a boy was healthy

The pregnancy had been normal the delivery was at term the birth weight was 3100 g No asphyxia or jaundice nor any abnormalities during the neonatal period were noted and the development had been normal

In 1965 the patient was admitted to an epidemic hospital for three weeks because of purulent meningitis (E coli) which was treated successfully with chemotherapeutics

Since birth the patient had been suffering from constipation for which she had been admitted to the surgical department of Glostrup Hospital four times between 1965 and 1967 A congenital anal stenosis was diagnosed and treated with dilatation of the anus In July 1967 the patient was transferred to the Department of Pediatrics because of hypersedimentatio (SR 123 mm/h) leukocyturia and bacteriuria

*Investigations* I v urography showed an uncertain reflux bilaterally and slight delay in the excretion of contrast material in the left kidney Cysto urethrography with voiding showed reflux in the left side The bladder was dislocated anteriorly X ray of the sacrum showed partial absence of the sacral and the coccyx Lumbo sacral myelography with injection of air and Pantopaque (an oil soluble contrast material (Fig 3) supplemented by X ray of the colon revealed a sacral ventral meningocele with communication to the spinal canal dislocation of the rectum as well as a slight anal stenosis Blood urea was 0 22 g per liter By rectal exploration a round mass was palpated at the bottom of the sacral cavity behind the rectum

Biopsy of the mucosa and muscular tissue of the rectum showed no pathological findings

*Case no 2*

An 8 year old girl (090559 L p r g ) cousin of patient no 1 was born as no 1 of 2 children No 2 was a healthy boy The pregnancy was complicated by pre eclampsia The delivery was at term and the birth weight was 4200 grams No asphyxia or jaundice was noted

Since birth the patient had suffered from constipation for which she had been hospitalized thrice from 1962 to 1967 A congenital anal stenosis was treated with dilatation of the anus in 1959 The second admission in 1963 was prompted by severe constipation with imminent ileus Anticonstipationdiet and various laxatives were given without result In January 1967 the patient was admitted to the Department of Pediatrics because of constipation and nocturnal enuresis

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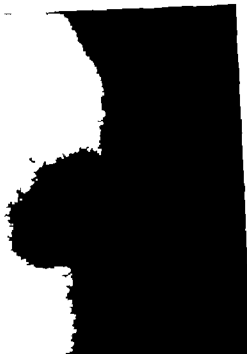


*Fig 4* A sagittal tomogram of the sacral region showing the air filled meningocele communicating with the spinal canal (air myelography) (case no 2)

*Case no 3*

A 30 year old woman (010338 W j e) mother of patient no 1 was no 11 of 14 siblings four of whom had died no 1 a boy one year old of tuberculosis no 4 a boy one year old of a thrombus in the intestine no 9 a girl one year old of meningitis no 10 a boy one month old of meningitis

At the age of one a congenital anal stenosis was diagnosed and treated with dilation of the anus with fair results The patient had been pregnant six times including one abortion

In 1966 an intravenous urography was made revealing a double kidney and double ureter in the right side At the same time partial absence of the sacrum and the coccyx was found Hystero-salpingography showed uterus bicornis (Fig 5) In 1966 a hysterectomy was performed because of hyperplasia of the uterus Clinically the patient was now healthy with



*Fig 5* Hysterosalpingogram showing bicornous uterus (case no 3)



*Fig 3* Lateral view of the lumbo sacral region The Pantopaque material is visible in the spinal cord and in the meningocele anteriorly to the sacrum (case no 1)

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the midline toward the merum behind the rectum probably a ventral meningocele

Plasma creatinine was 16 m per h er in 1961 increasing to 73 in 1966

## DISCUSSION

Sacral defects associated with ventral meningocele give rise to the following symptoms which may be divided into three major groups

1 *Pressure symptoms from the meningocele*
   a Rectum constipation
   b Urinary bladder incontinence of urine or enuresis residual urine recurrent infections of the urinary tract and localized pains
   c Internal genitals dysmenorrhoea dyspareunia labor difficulties (particularly in the expulsive stage)
2 *Neurological symptoms* disturbance of sphincter control anesthesia in the sacral region pains in the uro genital region motor weakness and paresthesia of the legs
3 Headache and nausea due to intermittent *increase in intracranial pressure*

The diagnosis can be established on clinical grounds however experience shows that it is difficult many erroneous diagnoses have been made or the disease may have been overlooked Radiologically the diagnosis can be made from an ordinary front view radiogram of the sacrum which shows a sickle shaped well-defined bone defect which is pathognomonic for the disease

The meningocele is best shown by air or Pantopaque myelography which reveals the presence of the meningocele and at the same time gives an idea of its dimensions Supplemental X ray studies of the bladder and the rectum with contrast material indicate its relations to the adjacent organs in the minor pelvis

*Differential diagnoses* include presacral dermoid cyst lipoma chondroma teratoma chordoma plasmocytoma neurinoma ovary tumor retrouterine hematocele and pelvic ec (10 13 16)

The prognosis for life is good in the absence of surgery Some workers advise the application of Cesarean section at deliveries in order to avoid the dangers of rupture of the meningocel and of increasing intracranial pressure during the expulsive stage The meningocele very rarely obstructs the birth canal a first described by Leigh et al in 1954 (24) Due to the frequency of infections of the urinary tract some of the patients mentioned in the literature died of chronic pyelonephritis with uremia Cases of meningitis have been reported most of which were caused by erroneous operative treatment (30)

The *therapy* is principally symptomatic particularly aiming at intensive treatment of the recurrent infections of the urinary tract The treatment of constipation can be extremely difficult and daily enemas may be necessary

Surgical therapy with puncture of the meningocele sometimes followed by aspiration has been used in the past however was frequently complicated by meningitis Ligature of the stalk excision or extirpation of the meningocele by laparotomy (22) parasacral incision or posterior incision (1 17) have involved a high mortality primarily due to postoperative meningitis

In 1940 Sherman et al (30) reported 26 cases 11 of whom died under or immediately after operation The remaining 15 patients died at a later time of postoperative meningitis

Leigh et al (24) reported on two patients who underwent radical surgery successfully (the stalk was ligated and the meningocele was extirpated) Silvis et al (31) mention one patient being operated upon with favorable results Jones & Evans (24) described one case where the patient expired shortly after the operation Barlow & Gracey (2) published another case of successful surgery So did Henley & Lawrence (19) Gurot et al (14) reported on a case where the meningocele was extirpated by laparotomy with favorable effect

Along with the improvement of surgical techniques and the appearance of chemotherapeutics and antibiotics the operative mortality has decreased out of seven patients operated on in the period from 1934 to 1966 six survived the operation



*Fig 6* Hysterosalpingography reveals uterus didelphys The hook shaped sacral defect is suggested (case no 4)

out constipation or symptoms from the urinary tract Plasma creatinine was 8 mgs per liter

*Case no 4*

A 27 year old woman (181240 L i g) mother of patient no 2 was no 14 of 14 siblings At birth she had hypoplasia of the left leg which was 4 cms shorter than the right and she also had pes equino varus in the left side

Ever since birth she had been constipated and in periods she had incontinence of feces There had been persistent incontinence of urine and because of recurrent infections of the urinary tract she had developed a chronic pyelonephritis In 1958 partial absence of the sacral and coccygeal bones and sacral spina bifida was diagnosed Myelography with injection of Conturex (a water soluble contrast material) showed a large sacral ventral meningocele

*Gynecological history* The patient had had two deliveries At both there were difficulties with the separation of the placenta The first child a girl was our patient no 2 the second a healthy boy Hysterosalpingography revealed uterus didelphys (Fig 6)

Plasma creatinine was 7 mgs per liter blood urea 0 12 grams per liter Microscopy of the urine showed leukocytes and bacteria

*Case no 5*

A 45 year old woman (280722 T g e) a sister of patients nos 3 and 4 was no 6 of 14 children She had had one abortion and given birth to four children All deliveries were uneventful Nos 1 and 2 both boys were healthy no 3 a girl now 13 years old still had incontinence of urine and feces No 4 a girl was healthy

Plasma creatinine was 9 mg per liter

Intravenous urography showed no abnormalities

*Case no 6*

A woman (031297 F m s died 30/4 1966) was the mother of patients nos 3 4 and 5 One of her sisters had diabetes mellitus

*Gynecological history* The patient had had no abortions 14 deliveries All pregnancies were complicated by pre eclampsia and a few by eclampsia At all deliveries the passage of the child through the birth canal was difficult and the expulsive labor was very weak Four children had died (cf case no 3)

In 1929 in connexion with a delivery a diagnosis of herniation of the spinal cord was made The patient at that time complained of incontinence of feces A puncture of the meningocele was performed without success Since 1950 there had been gradual progression of clinical symptoms of syringomyelia from both arms and legs including cutaneous analgesia and thermo anesthesia of both arms later also hypesthesia and paresthesia and progressive paresis She also had pains in the legs incoordination of the left leg and paresthesias in the left foot

In 1952 partial absence of the sacral and the coccygeal bones was found by accident at radiography

The patient had had diabetes mellitus since 19[illegible] and was treated with insulin Since 1954 there had been recurrent infections of the urinary tract and from 1961 also incontinence of feces Gynecological examination in 1965 revealed a rounded soft mass in

the midline toward the sacrum behind the rectum probably a ventral meningocele

Plasma creatinine was 16 mg per liter in 1961 increasing to 25 in 1966

## DISCUSSION

Sacral defects associated with ventral meningocele give rise to the following symptoms which may be divided into three major groups

1 *Pressure symptoms from the meningocele*
   a Rectum constipation
   b Urinary bladder incontinence of urine or enuresis residual urine recurrent infections of the urinary tract and localized pains
   c Internal genitals dysmenorrhoea dyspareunia labor difficulties (particularly in the expulsive stage)
2 *Neurological symptoms* disturbance of sphincter control anesthesia in the sacral region pains in the uro genital region muscular weakness and paresthesia of the legs
3 Headache and nausea due to intermittent *increase in intracranial pressure*

The *diagnosis* can be established on clinical grounds however experience shows that it is difficult many erroneous diagnoses have been made or the disease may have been overlooked Radiologically the diagnosis can be made from an ordinary front view radiogram of the sacrum which shows a sickle shaped well defined bone defect which is pathognomonic for the disease

The meningocele is best shown by air or Pantopaque-myelography which reveals the presence of the meningocele and at the same time gives an idea of its dimensions Supplemental X ray studies of the bladder and the rectum with contrast material indicate its relations to the adjacent organs in the minor pelvis

*Differential diagnoses* include presacral dermoid cyst lipoma chondroma teratoma chordoma plasmocytoma neurinoma ovary tumor retrouterine hematocele and pelvic echinococcus (7 10 13 16)

The *prognosis* for life is good in the absence of surgery Some workers advise the application of Cesarean section at deliveries in order to avoid the dangers of rupture of the meningocele and of increasing intracranial pressure during the expulsive stage The meningocele very rarely obstructs the birth canal as first described by Leigh *et al* in 1954 (24) Due to the frequency of infections of the urinary tract some of the patients mentioned in the literature died of chronic pyelonephritis with uremia Cases of meningitis have been reported most of which were caused by erroneous operative treatment (30)

The *therapy* is principally symptomatic particularly aiming at intensive treatment of the recurrent infections of the urinary tract The treatment of constipation can be extremely difficult and daily enemas may be necessary

Surgical therapy with puncture of the meningocele sometimes followed by aspiration has been used in the past however was frequently complicated by meningitis Ligature of the stalk excision or extirpation of the meningocele by laparotomy (22) parasacral incision or posterior incision (1 17) have involved a high mortality primarily due to postoperative meningitis

In 1950 Sherman *et al* (30) reported 26 cases 11 of whom died under or immediately after operation The remaining 15 patients died at a later time of post operative meningitis

Leigh *et al* (24) reported on two patients who underwent radical surgery successfully (the stalk was ligated and the meningocele was extirpated) Silvis *et al* (31) mention one patient being operated upon with favorable results Jones & Evans (2?) described one case where the patient expired shortly after the operation Barlow & Gracey (2) published another case of successful surgery So did Henley & Lawrence (19) Oncot *et al* (16) reported on a case where the meningocele was extirpated by laparotomy with favorable effect

Along with the improvement of surgical techniques and the appearance of chemotherapeutics and antibiotics the operative mortality has decreased out of seven patients operated on in the period from 1954 to 1966 six survived the operation



*Fig 6* Hysterosalpingography reveals uterus didelphys The hook shaped sacral defect is suggested (case no 4)

out constipation or symptoms from the urinary tract Plasma creatinine was 8 mgs per liter

*Case no 4*

A 27 year old woman (181240 L i g) mother of patient no 2 was no 14 of 14 siblings At birth she had hypoplasia of the left leg which was 4 cms shorter than the right and she also had pes equino varus in the left side

Ever since birth she had been constipated and in periods she had incontinence of feces There had been persistent incontinence of urine and because of re current infections of the urinary tract she had developed a chronic pyelonephritis In 1958 partial absence of the sacral and coccygeal bones and sacral spina bifida was diagnosed Myelography with injection of Conturex (a water soluble contrast material) showed a large sacral ventral meningocele

*Gynecological history* The patient had had two deliveries At both there were difficulties with the separation of the placenta The first child a girl was our patient no 2 the second a healthy boy Hysterosalpingography revealed uterus didelphys (Fig 6)

Plasma creatinine was 7 mgs per liter blood urea 0 12 grams per liter Microscopy of the urine showed leukocytes and bacteria

*Case no 5*

A 45 year old woman (280722 T g e) a sister of patients nos 3 and 4 was no 6 of 14 children She had had one abortion and given birth to four children All deliveries were uneventful Nos 1 and 2 both boys were healthy no 3 a girl now 13 years old still had incontinence of urine and feces No 4 a girl was healthy

Plasma creatinine was 9 mg per liter

Intravenous urography showed no abnormalities

*Case no 6*

A woman (031297 F m s died 30/4 1966) was the mother of patients nos 3 4 and 5 One of her sisters had diabetes mellitus

*Gynecological history* The patient had had no abortions 14 deliveries All pregnancies were complicated by pre eclampsia and a few by eclampsia At all deliveries the passage of the child through the birth canal was difficult and the expulsive labor was very weak Four children had died (cf case no 3)

In 1928 in connection with a delivery a diagnosis of herniation of the spinal cord was made The patient at that time complained of incontinence of feces A puncture of the meningocele was performed without success Since 1950 there had been gradual progression of clinical symptoms of syringomyelia from both arms and legs including cutaneous analgesia and thermo anesthesia of both arms later also hypesthesia and paresthesia and progressive paresis She also had pains in the legs incoordination of the left leg and paresthesias in the left foot

In 1952 partial absence of the sacral and the coccygeal bones was found by accident at radiography

The patient had had diabetes mellitus since 1954 and was treated with insulin Since 1954 there had been recurrent infections of the urinary tract and from 1961 also incontinence of feces Gynecological examination in 1965 revealed a rounded soft

the midline toward the sacrum behind the rectum probably a ventral meningocele

Plasma creatinine was 16 mg per liter in 1961 increasing to 25 in 1966

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M SEIP M HARBOE and K CYVIN

*From the Department of Pediatrics Rikshospitalet and the Institute for Experimental Medical Research Ullevål Sykehus University of Oslo and the Department of Pediatrics Trondheim Sykehus Trondheim Norway*

*Acute* autoimmune hemolytic anemias with cold antibodies are not rare in children and may be elicited e g by mycoplasma infections and by certain viral infections At least one case of *chronic* autoimmune hemolytic anemia with cold antibodies has been described in childhood (2) but the incidence is thought to be very low before adulthood (6 22) Chronic autoimmune hemolytic anemias with warm antibodies are much more common and may occur even as early as during the first year of life

The purpose of the present paper is to report a case of chronic hemolytic anemia with cold antibodies beginning before four years of age Two other features are also remarkable in this case the occurrence of repeated aplastic crises in connection with severe hemolytic episodes and the occurrence of autoimmune hemolytic anemia in an older sister

## CASE REPORTS

*Case 1*

A S is a girl born March 9 1959 as the fourth of four siblings A sister born 1953 and a brother born 1966 are healthy A sister born 1954 has had autoimmune hemolytic anemia (case 2)

The patient fell acutely ill with grave anemia in February 1963 and was admitted to Trondheim Hospital on February 21 1963 Hemoglobin was then 3 7 g/100 ml the direct Coombs test strongly positive and a pathological cold agglutinin was found in serum (titer 1 8) There were marked signs of hemolysis and increased red cell production The bone marrow showed signs of very active erythropoiesis and reticulocytosis and nucleated red cells were found in the peripheral blood Following treatment with prednisone and blood transfusions she went into complete remission although the Coombs test remained positive The medication was stopped and she was discharged home on April 5 1963

A week later she had an acute relapse and was readmitted to hospital Her hemoglobin dropped to 3 4 g serum bilirubin was 2 0 mg/100 ml This time reticulocytopenia with only 0 1 per cent reticulocytes in the peripheral blood was found despite the grave anemia Following blood transfusions and corticosteroids a reticulocytosis occurred and she went into another remission

She was kept on prednisone 7.5–10 mg daily and was in good remission until December 1963 when the medication was stopped A month later her symptoms recurred Cortico steroids had to be reinstituted

In March 1965 another attempt was made to withdraw the medication but a month later she was severely anemic with reticulocytopenia and corticosteroids had to be given She was well although with retardation of growth until March 1966 A new attempt to postpone the steroids was followed by relapse

She was admitted to the Department of Pediatrics Rikshospitalet Oslo in August 1967 She was then on prednisone 20 mg every other day and in good control A gradual withdrawal of the medication was attempted once more and she was discharged at the end of September with only mild signs of hemolysis

Soon afterwards she began to feel tired and on November 3 1967 she had to be readmitted to hospital with severe anemia due to strong hemolysis combined with a prolonged aplastic crisis The prednisone dose was increased to 10 mg three times daily but the hemolysis and red cell aplasia persisted Frequent blood transfusions had to be given Reticulocytes varied between 0 1 and 0 7 per cent most often 0 1 per cent The bone marrow showed marked hypoplasia of the erythroid elements red cell precursors

## SUMMARY

Six familial cases of partial absence of the sacrum and the coccyx are described Four had sacral ventral meningocele There was a sex linked dominant inheritance The diagnosis of the meningocele was established by myelography The symptoms were constipation and incontinence of urine Three patients also had recurrent infections of the urinary tract and three had congenital anal stenosis

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## DISCUSSION

Autoimmune hemolytic anemia with cold antibodies in children is as a rule an acute self limited illness provoked by infections with mycoplasma, mononucleosis virus or certain other viruses (22) The cold agglutinin titers are usually moderate but the antibodies have a marked ability to fix complement to red cells which results in decreased life span Immunoelectrophoresis shows increased concentration of γM globulin of the polyclonal type

The typical chronic cold hemagglutinin disease with hemoglobinuria and Raynaud's phenomena has not been described in childhood This disease is most common in elderly people The antibodies are present in very high titers belong to the γM globulins and are in many—perhaps all—cases monoclonal in origin (4 13 14) In cases of autoimmune hemolytic anemia secondary to collagen diseases (disseminated lupus erythematosus rheumatoid arthritis) or malignancies the antibody may occasionally be of the cold type (6) Another disease caused by a different kind of antibody active in the cold is paroxysmal cold hemoglobinuria syphilitic or non syphilitic All these disorders can be excluded in our case 1

Dacie (6) has studied four adult patients with what he calls less typical chronic autoimmune hemolytic anemia of the cold antibody type distinct from the cold hemagglutinin disease None of these had Raynaud's phenomena and only one had had an episode with hemoglobinuria They gave positive antiglobulin tests of the non γ type This reaction pattern is caused by complement absorbed to the red cell surface in vivo (15) The cold agglutinin titers were only moderately increased to 256–1024 These patients in addition had an antibody causing agglutination and particularly hemolysis of trypsinized erythrocytes at 37 C The serological findings in our case 1 are very similar

Bonham-Carter *et al* (2) have reported the case of a five year-old boy who developed chronic autoimmune hemolytic anemia with cold antibodies of high thermal amplitude following an upper respiratory infection The clinical picture was similar to that of our patient A S No details concerning the cold agglutinin are given but the patient may have had the same or a similar disorder as A S To our knowledge this is the only convincing observation of idiopathic chronic autoimmune hemolytic anemia with cold antibodies in childhood published so far The disorder must be very rare but not necessarily as rare as the literature may suggest In many published cases of acquired hemolytic anemia in childhood the serologic evaluation has been incomplete

It is conceivable that the autoimmune hemolytic anemia in our patient 1 and in the patient of Bonham-Carter has started as an acute process in response to a mycoplasma or viral infection and that for one reason or another (genetic predisposition?) the process has not shown the usual self limited course but has developed into a chronic disease The serologic findings are compatible with this hypothesis which however is difficult to prove beyond doubt at the present stage

It is well known that several autoimmune diseases may show familial occurrence and that genetic factors may play a role in their etiology (3) This is particularly striking for Hashimoto's thyroiditis Systemic lupus erythematosus and rheumatoid arthritis may also show familial occurrence Among apparently healthy relatives of patients with autoimmune disorders the incidence of aberrations in the serum γ globulins is higher than normal (12) In hereditary hypogammaglobulinemias and dysgammaglobulinemias the incidence of autoimmune disorders is definitely increased

Familial autoimmune hemolytic anemia has been described in only a few instances We have found nine convincing reports in the literature either with occurrence of the disease in mother and daughter (5 17) mother and son (12 23) or in siblings (9 16 18 19) In all these reported cases in contrast to ours the onset of the hemolytic anemia has been in

amounting to only 1 per cent of the bone marrow cells During this episode two white cell counts as low as 2300 and 2400 were registered but the differential counts were normal Otherwise all white blood cell and platelet counts have been normal

On December 15 she was started on prednisone 15 mg four times daily and methandrostenolone 5-7 5 mg daily After about a week a gradual improvement took place She received her last blood transfusion on December 20 A slight increase in reticulocytes was noticed from December 24 and a peak of 11 3 per cent was reached on January 1 1968 By January 21 hemoglobin red cells and reticulocytes were all normal Methandrostenolone was postponed January 17

To reduce her steroid requirement splenectomy was performed on February 23 1968 The spleen weighed 120 g On microscopic examination signs of increased red cell destruction were found After the operation she still seemed to need 20 mg of prednisone per day From March 29 she has therefore received azothioprine 25 mg three times daily in addition to prednisone in an attempt to reduce the prednisone dose Her hemoglobin values are now stable around 10 5 g and her reticulocyte counts only slightly elevated (2 0 2 5 per cent) on azothioprine 75 mg daily and prednisone 20 mg in one dose every 48 hrs

The patient has never had hemoglobinuria or *Raynaud s phenomena*

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## DISCUSSION

Autoimmune hemolytic anemia with cold antibodies in children is as a rule an acute self limited illness provoked by infections with mycoplasma, mononucleosis virus or certain other viruses (22). The cold agglutinin titers are usually moderate but the antibodies have a marked ability to fix complement to red cells which results in decreased life span. Immunoelectrophoresis shows increased concentration of γM globulin of the polyclonal type.

The typical chronic cold hemagglutinin disease with hemoglobinuria and Raynaud's phenomena has not been described in childhood. This disease is most common in elderly people. The antibodies are present in very high titers belong to the γM globulins and are in many—perhaps all—cases monoclonal in origin (4, 13, 14). In cases of autoimmune hemolytic anemia secondary to collagen diseases (disseminated lupus erythematosus, rheumatoid arthritis) or malignancies the antibody may occasionally be of the cold type (6). Another disease caused by a different kind of antibody active in the cold is paroxysmal cold hemoglobinuria, syphilitic or non syphilitic. All these disorders can be excluded in our case 1.

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It is well known that several autoimmune diseases may show familial occurrence and that genetic factors may play a role in their etiology (3). This is particularly striking for Hashimoto's thyroiditis. Systemic lupus erythematosus and rheumatoid arthritis may also show familial occurrence. Among apparently healthy relatives of patients with autoimmune disorders the incidence of aberrations in the serum γ globulins is higher than normal (12). In hereditary hypogammaglobulinemias and dysgammaglobulinemias the incidence of autoimmune disorders is definitely increased.

Familial autoimmune hemolytic anemia has been described in only a few instances. We have found nine convincing reports in the literature either with occurrence of the disease in mother and daughter (5, 17), mother and son (12, 23) or in siblings (9, 16, 18, 19). In all these reported cases, in contrast to ours, the onset of the hemolytic anemia has been in

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adulthood or in adolescence (16) It could be mentioned that a strain of inbred mice is known where genetically determined hemolytic anemia with positive direct Coombs test and other autoimmune features consistently develops within some months (1)

Shapiro (21) has reported a family in which five siblings died from a peculiar disease picture with hemolytic anemia and signs of runting syndrome and in which the parents and the two living siblings also had an abnormal serum gamma globulin pattern In two of these patients the Coombs test was performed and found to be positive Only one of them was studied in detail She had a $Rh_0$ specific autoantibody hypergammaglobulinemia and thymic atrophy and generalized lymphocytic depletion at autopsy The author suggests that the autoimmune hemolytic anemia and other pathologic manifestations might be the result of a graft versus-host reaction This interesting disease is definitely quite different from that of our two patients

It is striking that the serological findings were *different in the presently described two* sisters with autoimmune hemolytic anemia In patient 1 cold agglutinins were found in the serum together with an antibody causing hemolysis of trypsinized red cells and only complement was detected on the red cells by the direct Coombs test Patient 2 had warm type hemolytic anemia with a $\gamma$G antibody on her red cells A simple direct inheritance of a specific type of autoimmune hemolytic anemia therefore does not seem to have taken place The observations indicate the presence of a more fundamental genetic aberration of the immune apparatus in these two siblings giving rise to serologically different forms of hemolytic anemia A parallel to this is that in families where a *predisposition to e g dissemi*nated lupus erythematosus is present there is also an increased incidence of gammaglobulin disturbances and other forms of autoimmune diseases

The occurrence of aplastic crises or phases in patients with autoimmune hemolytic anemia has been reported repeatedly since the earlier observations by Davis (8) Bonham Carter (2) and Seip (20) These episodes may be acute lasting for a few weeks only or more prolonged (2, 10)

In our case 1 all the more severe hemolytic episodes except the first, were accompanied by marked reticulocytopenia and by erythroblastopenia in the bone marrow (on the occasions when bone marrow puncture was performed) During such phases only 1 per cent of the bone marrow cells were red cell precursors During her more than five years of illness she has had five such aplastic episodes, the last one continuing for as long as eight weeks and finally responding to large doses of corticosteroids and anabolic steroids Such a marked tendency to erythroid aplasia is quite unique in autoimmune hemolytic anemia

It should be mentioned that the five year-old boy with chronic autoimmune hemolytic anemia and cold antibodies reported by Bonham Carter *et al* (2) also had two aplastic episodes one of which lasted for five months *These episodes too responded to large* doses of steroids On the whole the clinical pictures of our case 1 and the patient described by Bonham Carter are very similar As mentioned earlier Dacie (6) in his book on hemolytic anemias reports four atypical cases of chronic hemolytic anemia with cold antibodies who did not suffer from the ordinary cold hemagglutinin disease of adults One of these a 56 year-old woman described in detail by Dacie & de Gruchy (7) had reticulocytopenia with only 0 5 per cent reticulocytes during an episode of severe hemolysis and marked anemia It is possible that the risk of bone marrow aplasia is greater in autoimmune hemolytic anemia with cold antibodies than when warm antibodies are concerned

Most authors believe that the reason for the erythroid aplasia in these cases is that the red cell antibodies may damage even the red cell precursors in the bone marrow However the mechanism of the erythroid aplasia is still unclear Many observations indicate that cold ag

glutinins are active only at low temperatures. The in vitro agglutination caused by cold agglutinins is rapidly reversed at 37 C and so is the in vivo agglutination in small vessels occurring after cold exposure. It is therefore not so easy to explain how the cold agglutinins could exert a detrimental influence on the red cell precursors in the bone marrow. Anyway in our patient the most severe exacerbations of the hemolytic process have repeatedly been accompanied by red cell aplasia. Perhaps the demonstrated additional antibody causing hemolysis of trypsinized red cells at 37 C may have a detrimental effect on red cells and red cell precursors. This could also explain why the severity of the clinical picture of the patient did not seem to be related to exposure to cold.

It has not been established when antibodies which react in vitro at 37 C only with enzyme treated cells reduce the survival of normal red cells in vivo but Engelfriet et al (11) have recently proposed a new serological classification of autoimmune hemolytic anemias where patients with only warm hemolysins active against enzyme treated cells are included in a separate group.

## SUMMARY

A report is given of two sisters who developed chronic autoimmune hemolytic anemia in their fourth and twelfth years of age respectively. The serological findings were different in the two cases. In patient 1 a typical cold agglutinin with a titer of 256 at 4 C was found in the serum and only complement on the red cells by the direct Coombs test. The serum hemolysed trypsinized red cells at 37 C. Her sister had warm type hemolytic anemia with a γG antibody on her red cells. In case 1 the clinical course has been severe with several exacerbations of the hemolytic process accompanied by erythroid aplasia in the bone marrow.

Whereas *acute* autoimmune hemolytic anemia with cold antibodies is not rare in children and may be elicited by mycoplasma or viral infections, *chronic* autoimmune hemolytic anemia is rarely seen before adulthood. The typical chronic cold hemagglutinin disease with hemoglobinuria and Raynaud's phenomena has not been described in childhood and was not present in our case. It is suggested that the disease in the case presented may have started as the result of an acute infection and that for one reason or another (genetic predisposition?) the process has not shown the usual self limited course.

Although a familial—probably genetic—predisposition exists for several autoimmune diseases, familial occurrence of autoimmune hemolytic anemia has rarely been observed. The authors have found nine convincing reports in the literature and in only one of the reported families did the hemolytic anemia develop in childhood. In families predisposed to autoimmune diseases a variety of γ globulin disturbances and autoimmune disorders are often found. The presence of a more fundamental genetic aberration of the immune apparatus preventing a normal immune homeostasis may therefore be postulated.

A brief comment is made on the possible mechanism of the repeated periods of erythroid aplasia in the patient with cold antibodies. It is suggested that a second antibody causing hemolysis of trypsinized red cells at 37 C may be of importance by damaging the red cell precursors. The presence of this antibody may also possibly explain why the severity of the clinical picture of the patient did not seem to be related to exposure to cold.

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## SUMMARY


A report is given of two sisters who developed chronic autoimmune hemolytic anemia in their fourth and twelfth years of age respectively The serological findings were different in the two cases In patient 1 a typical cold agglutinin with a titer of 256 at 4 C was found in the serum and only complement on the red cells by the direct Coombs test The serum hemolysed trypsinized red cells at 37 C Her sister had warm type hemolytic anemia with a γG antibody on her red cells In case 1 the clinical course has been severe with several exacerbations of the hemolytic process accompanied by erythroid aplasia in the bone marrow

Whereas acute autoimmune hemolytic anemia with cold antibodies is not rare in children and may be elicited by mycoplasma or viral infections chronic autoimmune hemolytic anemia is rarely seen before adulthood The typical chronic cold hemagglutinin disease with hemoglobinuria and Raynaud's phenomena has not been described in childhood and was not present in our case It is suggested that the disease in the case presented may have started as the result of an acute infection and that (for one reason or another (genetic predisposition?) the process has not shown the usual self limited course

Although a familial—probably genetic—predisposition exists for several autoimmune diseases familial occurrence of autoimmune hemolytic anemia has rarely been observed The authors have found nine convincing reports in the literature and in only one of the reported families did the hemolytic anemia develop in childhood In families predisposed to autoimmune diseases a variety of γ globulin disturbances and autoimmune disorders are often found The presence of a more fundamental genetic aberration of the immune apparatus preventing a normal immune homeostasis may therefore be postulated

A brief comment is made on the possible mechanism of the repeated periods of erythroid aplasia in the patient with cold antibodies It is suggested that a second antibody causing hemolysis of trypsinized red cells at 37 C may be of importance by damaging the red cell precursors The presence of this antibody may also possibly explain why the severity of the clinical picture of the patient did not seem to be related to exposure to cold


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*Acta Pædiat Scand 58* 281–286 1969

# THE EFFECT OF ANTIMICROBIAL AGENTS ON THE BINDING OF BILIRUBIN BY ALBUMIN

KETI MALAKA ZAFIRIU and BASIL S STRATES

*From the Clinic of Pediatrics and the Laboratory of Pharmaceutical Chemistry University of Thessaloniki Greece*

The distribution of bilirubin in intravascular and extravascular fluids depends on a number of important variables one of which is the binding capacity of serum albumin The amount of albumin bound bilirubin in the blood is determined by the sites available for binding bilirubin to albumin Availability of these sites varies mainly with albumin concentration pH and the presence of drugs or other substances exhibiting chemical affinity for albumin (6)

Silverman *et al* (10) observed a higher incidence of deaths among premature infants treated with sulfisoxazole than in controls receiving oxytetracycline They further noted large extravascular quantities of bilirubin associated with relatively low serum bilirubin levels in the sulfonamide treated group *In vitro* experiments (4 5 7) indicate that sulfonamides shift bilirubin from plasma to the extravascular fluid by competing for the serum albumin binding sites

More recently Boe *et al* (1) have reported on the serum concentrations of methicillin and ampicillin administered to term and premature newborns Hyperbilirubinemic term infants showed higher methicillin levels than the average for their age group but it could not be established whether this was due to displacement of albumin bound methicillin by bilirubin or to decreased hepatic function

The binding capacity of serum albumin in normal and jaundiced newborns has been determined by a dialysis technique employing the dye phenolsulfonphthalein (PSP) (2 13) It was found that the amounts of PSP bound are inversely proportional to the serum concentrations of bilirubin or certain ions such as those of hematin sodium salicylate and sulfisoxazole competing for the binding sites of albumin Icteric sera from newborn infants with bilirubin encephalopathy showed little or no PSP binding capacity (14)

To overcome the limitations of the PSP technique Porter & Waters (8) have modified the method of 2 (4 hydroxybenzeneazo) benzoic acid (HBABA) proposed by Rutstein *et al* (9) so as to measure available albumin binding sites instead of serum albumin concentrations The HBABA method is less time consuming and requires a small volume of serum It is better suited for use in the clinical laboratory than the PSP technique

The aim of the present work was (*a*) to examine to which degree certain antibiotics reduce the albumin binding capacity for HBABA and consequently for bilirubin and (*b*) to provide additional experimental data on the interference of these agents with the binding of bilirubin by serum albumin

## MATERIALS AND METHODS

The chemical reagents used in these experiments were crystalline human serum albumin (Sigma) analytical grade 2-(4 hydroxybenzeneazo) benzoic acid (HBABA K & K Laboratories) and chemically pure bilirubin (Merck Darmstadt) Antimicrobial agents sulfonamides and antibiotics were supplied by vari

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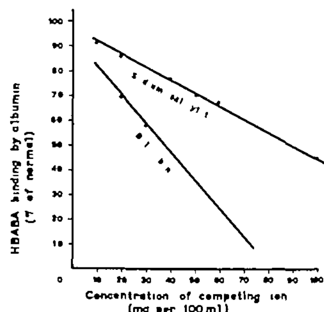



*Fig 1* Depression of albumin binding capacity (4 g albumin per 100 ml of solution) for HBABA dye in the presence of bilirubin (10–70 mg per 100 ml) and sodium salicylate (10–100 mg per 100 ml)

ous manufacturers of pharmaceutical products (Table 1)

Two solutions of albumin were prepared containing respectively 4 and 8 g crystalline reagent per 100 ml of 0 9 % NaCl Stock and working dye solutions were prepared and stored according to directions given in the literature (8) A fresh working dye solution was prepared at the end of every 10 to 20 days Solutions kept in the refrigerator were equilibrated at 25 ± 0 5 C before use The dilutions of bilirubin (10–70 mg per 100 ml) were made with the 4 g per 100 ml albumin solution

The procedure has been described in detail by other workers (8) For the present experiment test tubes were prepared as follows (1) *test mixture* 3 ml of working dye solution + 0 1 ml of solution containing 0 05 ml of albumin solution (8 gm per 100 ml) and 0 05 ml of drug solution (in distilled water or 0 9 % NaCl according to directions) (2) *dye blank* 3 ml of working dye solution + 0 1 ml of distilled water and (3) *albumin blank* 3 ml of 0 02 M Tris buffer solution (Sigma) + 0 1 ml of albumin solution (4 g per 100 ml)

Albumin concentrations were determined by the biuret method Bilirubin was measured by a modification of the Malley & Evelyn technique (3)

## RESULTS

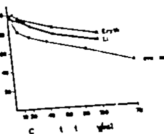
### *Bilirubin and sodium salicylate*

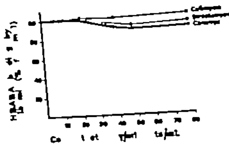
Before testing the binding of antimicrobial agents by albumin, the binding capacity of the 4 g per 100 ml albumin solution for the dye 2 (4 -hydroxybenzeneazo) benzoic acid (HBABA) was measured in the presence of bilirubin and sodium salicylate (Fig 1) For concentrations of bilirubin ranging from 10 to 70 mg per 100 ml a higher depression of albumin binding capacity was noted than with equal amounts of sodium salicylate added in the form of solutions containing 10 to 100 mg of drug per 100 ml

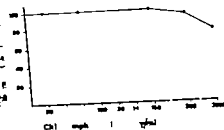
The slopes of the curves obtained indicate that sodium salicylate competes with the HBABA for the albumin binding sites but not as effectively as bilirubin Approximately 50 depression of albumin binding capacity for HBABA is brought about by concentrations of 90 mg of sodium salicylate per 100 ml of solution Therapeutic serum levels of salicylates often exceed the above value

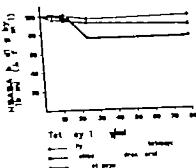
Table 1 *Depression of HBABA binding by albumin standard (4 g per 100 ml) at antibiotic concentrations similar to the therapeutic blood levels*

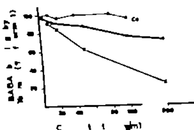
| *Generic name* | *Proprietary name and manufacturer* |
|---|---|
| *Depression 0–10* | |
| 1 Penicillin G sodium | Hoechst |
| 2 Methicillin sodium | (*a*) Staphcillin Bristol<br>(*b*) Celbenin Beecham |
| 3 Ampicillin | (*a*) Pentrexyl Bristol<br>(*b*) Penbritin Beecham |
| 4 Cloxacillin sodium | Orbenin Beecham |
| 5 Lincomycin hydrochlorid monohydrate | Lincocin Upjohn |
| 6 Cephaloridin | Ceporan Glaxo |
| 7 Streptomycin sulfate | Hoechst |
| 8 Kanamycin sulfate | Kantrex Bristol |
| 9 Colistin methanesulphonate | Colimycin Smit |
| 10 Chloramphenicol succinate | Syntomycetin Lepetit |
| 11 Erythromycin lactobionate | Erythrocin Abbot |
| 12 *Tetracyclin hydrochloride* | Signamycin Pfizer |
| 13 Pyrolidinomethyl tetracyclin | Bristacin Bristol |
| 14 Oxytetracyclin | Terramycin Pfizer |
| *Depression 11–25* | |
| 15 Oxacillin | Prostaphlin Bristol |
| 16 Cephalothin sodium | Keflin Lilly |
| 17 Rifamycin | Rifocin Lepetit |
| *Depression up to 25* | |
| 18 Novobiocin | Albamycin Upjohn |
| 19 Sulfisoxazole | Gantrisin Hoffman La Roche |
| 20 Sulfexin (long acting sulfonamide) | Sulfexin Adelco |
| 21 Sulfaisodimidin | Elkosin Ciba |

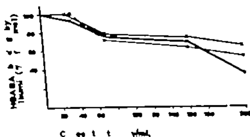


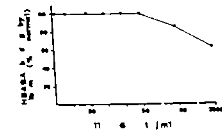


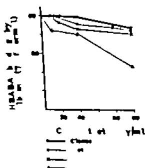












Figs 2–9. Depression of albumin binding capacity (4 g albumin per 100 ml of solution) for HBABA dye in the presence of the tested antimicrobial drugs at varied concentrations.

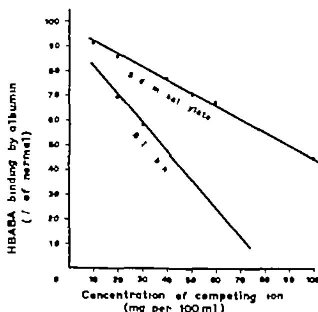



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## RESULTS

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| 7 Streptomycin sulfate | Hoechst |
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| 9 Colistin methanesulphonate | Colimycin Smit |
| 10 Chloramphenicol succinate | Syntomycetin Lepetit |
| 11 Erythromycin lactobionate | Erythrocin Abbot |
| 12 Tetracyclin hydrochloride | Signamycin Pfizer |
| 13 Pyrolidinomethyl tetracyclin | Bristacin Bristol |
| 14 Oxytetracyclin | Terramycin Pfizer |
| *Depression 11–25* | |
| 15 Oxacillin | Prostaphlin Bristol |
| 16 Cephalothin sodium | Keflin Lilly |
| 17 Rifamycin | Rifocin Lepetit |
| *Depression up to 25* | |
| 18 Novobiocin | Albamycin Upjohn |
| 19 Sulfisoxazole | Gantrisin Hoffman La Roche |
| 20 Sulfexin (long acting sulfonamide) | Sulfexin Adelco |
| 21 Sulfasodimidin | Elkosin Ciba |

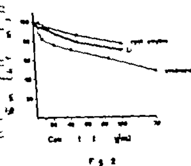


Fig. 2

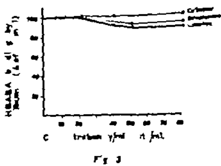


Fig. 3

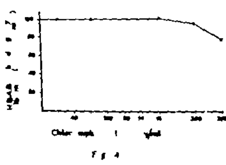


Fig. 4

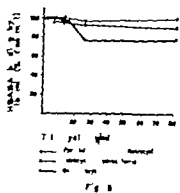


Fig. 5

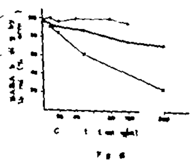


Fig. 6

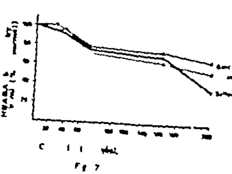


Fig. 7

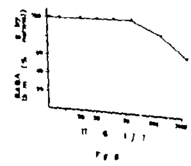


Fig. 8

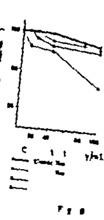


Fig. 9

Fig. 2–9. Depression of albumin binding capacity (4 g albumin per 100 ml of solution) for HBABA dye in the presence of the tested antimicrobial drugs at varied concentrations

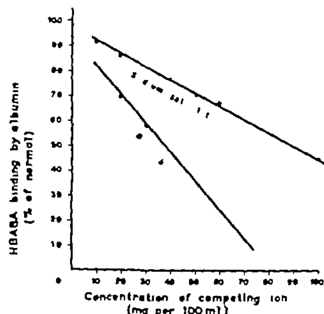



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## RESULTS

### *Bilirubin and sodium salicylate*

Before testing the binding of antimicrobial agents by albumin the binding capacity of the 4 g per 100 ml albumin solution for the dye 2 (4 -hydroxybenzeneazo) benzoic a[illegible] (HBABA) was measured in the pres[illegible] bilirubin and sodium salicylate (Fig 1) [illegible] concentrations of bilirubin ranging from [illegible] 70 mg per 100 ml a higher depression of [illegible]bumin binding capacity was noted than [illegible] equal amounts of sodium salicylate added [illegible] the form of solutions containing 10 to 100 [illegible] of drug per 100 ml

The slopes of the curves obtained [illegible] that sodium salicylate competes with [illegible] HBABA for the albumin binding sites but [illegible] as effectively as bilirubin Approximately [illegible] depression of albumin binding capacity [illegible] HBABA is brought about by concentration [illegible] 90 mg of sodium salicylate per 100 ml [illegible] solution Therapeutic serum levels of [illegible]cylates often exceed the above value

Table 1 *Depression of HBABA binding by al[illegible]min standard (4 g per 100 ml) at antibiotic con[illegible]centrations similar to the therapeutic blood le[illegible]*

| | *Generic name* | *Proprietary name and manufacturer* |
|---|---|---|
| *Depression 0–10* | | |
| 1 | Penicillin G sodium | Hoechst |
| 2 | Methicillin sodium | (a) Staphcillin Bri[illegible] (b) Celbenin Beech[illegible] |
| 3 | Ampicillin | (a) Pentrexyl Brist[illegible] (b) Penbritin Beecham |
| 4 | Cloxacillin sodium | Orbenin Beecham |
| 5 | Lincomycin hydrochlorid monohydrate | Lincocin Upjohn |
| 6 | Cephaloridin | Ceporan Glaxo |
| 7 | Streptomycin sulfate | Hoechst |
| 8 | Kanamycin sulfate | Kantrex Bristol |
| 9 | Colistin methanesulphonate | Colimycin Scot |
| 10 | Chloramphenicol succinate | Syntomycetin Lepeti[illegible] |
| 11 | Erythromycin lactobionate | Erythrocin Abbot |
| 12 | Tetracyclin hydrochloride | Signamycin Pfizer |
| 13 | Pyrolidinomethyl tetracyclin | Bristacin Bristol |
| 14 | Oxytetracyclin | Terramycin Pfi[illegible] |
| *Depression 11–25* | | |
| 15 | Oxacillin | Prostaphlin Bristol |
| 16 | Cephalothin sodium | Keflin Lilly |
| 17 | Rifamycin | Rifocin Lepetit |
| *Depression up to 25* | | |
| 18 | Novobiocin | Albamycin Upjohn |
| 19 | Sulfisoxazole | Gantrisin Hoffman La Roche |
| 20 | Sulfetrin (long acting sulfonamide) | Sulfetrin Adeko |
| 21 | Sulfamodimidin | Elkosin Ciba |

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## SUMMARY


The effect of antibiotics on the albumin binding capacity was studied by adding various concentrations of each drug in a standard solution of crystalline human serum albumin 4 g/100 ml according to method of Porter & Waters (8) We found significant depression (above 25 %) of binding capacity of the albumin after addition of the following drugs in concentrations reaching therapeutical blood levels Novobiocin and Sulfonamides (Elkosin Sulfexin Gantrisin)

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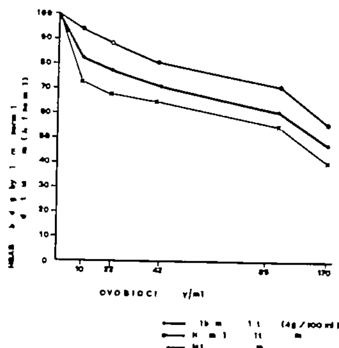


*Fig 10* Depression of HBABA binding by albumin standard normal adult serum and icteric serum of known binding capacity in the presence of Novobiocin (10–170 γ/ml)

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The above mentioned results are better shown in Figures 2–9 The slopes of the curves obtained indicate that many of the tested antimicrobial agents, added to the albumin standard solution in concentrations higher than the therapeutical blood levels, resulted in a significant depression of the albumin binding capacity for HBABA

Similar experiments conducted with normal adult serum and serum from jaundiced newborns, of known binding capacity for HBABA, showed that the addition of all the tested drugs resulted in a reduction of the serum albumin binding capacity for HBABA comparable to that of crystalline albumin solution (Fig 10)

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It is well known that many drugs, including antibiotics are reversibly bound by proteins in the serum as well as by proteins in the tissues with the result that *in vivo* the drug exists partly in the form of a protein bound complex and partly as the free compound The protein bound antibiotic in addition to being virtually inactive is also relatively non diffusible These qualities are related to the pharmacological problems the main problem however for the neonatal period is the competition of these drugs for the albumin binding sites for bilirubin Because these competing drugs may displace bilirubin from albumin, resulting in an increase in unbound bilirubin which is free to diffuse into the tissues especially in the brain

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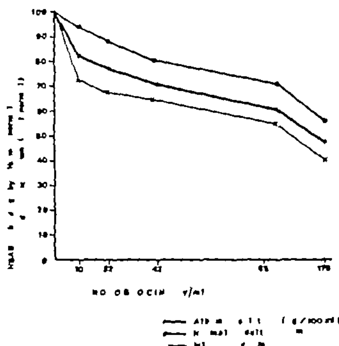


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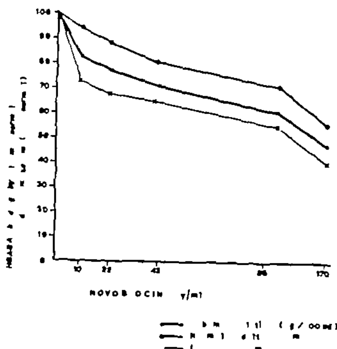


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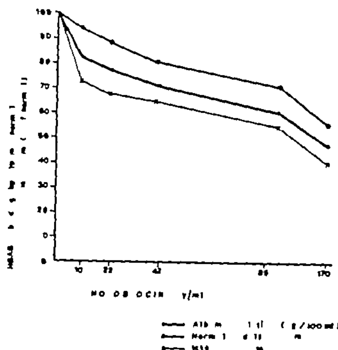


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Key words Albumin binding capacity antimicrobial agents

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# HAIR AMINO ACIDS IN CYSTINOSIS HOMOCYSTINURIA FÖLLING S DISEASE AND TYROSINOSIS

E J VELLAN L R GJESSING and M SEIP

*From Dikemark Hospital Asker and the Department of Pediatrics Rikshospitalet University of Oslo Oslo Norway*

In 1967 Schneider *et al* (4) found a large increase of cystine in leukocytes from patients with cystinosis This amino acid is also accumulated in the liver and the cerebrospinal fluid (5) and even deposited as crystals in the cornea and the bone marrow We therefore studied the composition of the amino acids in the hair from two children with cystinosis and from their mother as well as from two normal controls and from patients with homocystinuria Fölling s disease (phenylketonuria) and tyrosinosis

## MATERIALS AND METHODS

The material consisted of hair from two brothers with cystinosis (5) their mother one patient with homocystinuria one with tyrosinosis one with Fölling s disease and two normals The hair was first washed with destilled water then with light petroleum ethanol and ether 10 mg dried hair was hydrolyzed for 6 hours at 100 C in a sealed tube with 1 ml 6 N hydrochloric acid The hydrolysate was then evaporated to dryness in a stream of air at 40 C and dissolved in 1 ml 0 1 N hydrochloric acid 50 µl of this solution was chromatographed on a Technicon Amino Acid Auto Analyzer according to our previous description (5) and measured by Technicon Integrator Calculator In order to compare the content of the amino acids in the hair the values of each amino acid are expressed as g nitrogen per 100 g nitrogen recovered from column (ammonia excluded)

## RESULTS

The results of our amino acid analysis on the hair from 8 different subjects are listed in Table I

The content of *methionine* in the hair was the same for the patients with cystinosis and their mother as well as for the patient with tyrosinosis The methionine level was somewhat lower in homocystinuria and in Fölling s disease and lowest in our two normal controls

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*Tyrosine* and *phenylalanine* levels in tyrosinosis and Fölling s disease were within the range of the two normals No *homocystine* was found in homocystinuria

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i two normal controls No significant increases of cystine homocystine methionine phenyl alanine or tyrosine were found Analysis of hair amino acids therefore does not seem to give any information about the diagnosis in these disorders

## ACKNOWLEDGEMENTS

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Key words Hair amino acids cystinosis homocystinuria phenylketonuria tyrosinosis

Table 1 *Amino acid analysis of hair*

Results expressed as g nitrogen per 100 g nitrogen recovered from column (ammonia excluded)

| Disease | Cystinosis AK | Cystinosis TK | Mother of cystinosis | Homo cystinuria EA | Folling s disease TH | Tyro-sinosis AH | Control SEV | Control RL |
|---|---|---|---|---|---|---|---|---|
| Age in yrs | 6 | 4 | 28 | 3 | 34 | 5 | 3 | 46 |
| Hair color | Grey blond | Grey blond | Yellow blond | Yellow blond | Dark blond | Dark blond | Blond | Dark blond |
| Cysteic acid | 0 13 | 0 12 | 0 69 | 0 28 | 0 17 | 0 12 | 0 10 | 0 09 |
| Aspartic acid | 3 82 | 4 04 | 4 20 | 4 49 | 4 44 | 4 19 | 3 91 | 4 55 |
| Threonine | 4 77 | 5 25 | 5 04 | 5 21 | 5 16 | 5 29 | 5 06 | 6 09 |
| Serine | 10 29 | 10 59 | 10 16 | 9 99 | 10 12 | 9 76 | 10 02 | 9 90 |
| Glutamic acid | 9 67 | 9 11 | 9 81 | 10 19 | 9 93 | 9 61 | 9 60 | 9 87 |
| Proline | 7 05 | 4 10 | 5 75 | 5 62 | 6 07 | 3 34 | 4 40 | 4 53 |
| Citrulline | 0 38 | 0 29 | 0 45 | 0 09 | 0 33 | 0 27 | 0 24 | 0 14 |
| Glycine | 4 85 | 5 03 | 5 21 | 5 32 | 5 18 | 4 94 | 4 93 | 4 66 |
| Alanine | 3 60 | 3 69 | 3 76 | 4 02 | 3 95 | 3 71 | 3 41 | 3 03 |
| Valine | 2 74 | 2 69 | 3 25 | 3 01 | 2 84 | 2 80 | 2 43 | 2 93 |
| Cystine | 22 18 | 23 10 | 19 57 | 16 68 | 19 02 | 22 93 | 23 58 | 22 65 |
| Methionine | 0 28 | 0 29 | 0 27 | 0 21 | 0 21 | 0 30 | 0 10 | 0 09 |
| Isoleucine | 1 19 | 1 11 | 1 31 | 1 45 | 1 22 | 1 20 | 1 10 | 1 79 |
| Leucine | 4 59 | 5 27 | 5 09 | 5 33 | 4 80 | 5 43 | 5 51 | 4 80 |
| Tyrosine | 0 88 | 1 44 | 1 37 | 1 45 | 1 40 | 1 54 | 1 61 | 1 49 |
| Phenylalanine | 1 20 | 1 09 | 1 09 | 1 53 | 1 26 | 1 13 | 1 16 | 1 71 |
| Ornithine | Trace | 0 70 | | | 0 19 | 0 18 | | Trace |
| Lysine | 3 71 | 3 82 | 4 02 | 3 79 | 3 63 | 3 64 | 2 74 | 3 73 |
| Histidine | 1 80 | 1 76 | 1 81 | 1 83 | 2 12 | 1 86 | 2 08 | 1 65 |
| Arginine | 16 88 | 16 54 | 17 15 | 19 50 | 17 97 | 17 76 | 18 04 | 16 [illegible]0 |

## DISCUSSION

Pollitt *et al* (2) found a cystine abnormality in hair from two sibs with mental and physical retardation as well as trichorrhexis nodosa The hair from these two patients had a cystine content of only about one third of our normal values and about half of their normal value The cystine values were normal in hair from our two brothers with cystinosis and from their mother The low hair cystine level in the patient with homocystinuria may be due to the low level of this amino acid in the blood

It has been suggested that excess of phenylalanine may result in the production of abnormal protein by altering the synthetic processes through increased incorporation of phenylalanine into the protein molecule Earlier investigations (1) however showed no abnormalities in the pattern of the amino acids in the protein from various phenylketonuric organs Our results fit well with these findings as the phenylalanine content in hair from a patient with Folling s disease was normal In tyrosinosis we did not find an elevated level o hair tyrosine and in homocystinuria there wa no trace of homocystine in the hair

The small differences which we have foun in cystine methionine cysteic acid citrullin and proline may be within the normal rang This range cannot be firmly established unt a greater number of normal controls have bee studied

All the other hair amino acid values seem t be within the normal range in the patients wit cystinosis Folling s disease, tyrosinosis an homocystinuria

This study shows that the well known amin acid anomalies in these diseases are not mi rored in the keratin protein of the hair excep perhaps for a relatively low cystine content i homocystinuria

## SUMMARY

The amino acid content of the hair was deter mined in patients with cystinosis homocystin uria phenylketonuria and tyrosinosis and i

able 1 *Serum electrophoresis at 1 month interval without treatment*

| J M | Total proteins g/100 ml | | Albumins g/100 ml | | Alfa 1 globulins g/100 ml | | Alfa 2 globulins g/100 ml | | Beta globulins g/100 ml | | Gamma globulins g/100 ml | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| st determination | 8 6 | 100 | 4 28 | 49 80 | 0 33 | 3 88 | 0 74 | 8 57 | 0 70 | 8 10 | 2 55 | 29 60 |
| nd determination | 7 8 | 100 | 4 09 | 52 40 | 0 36 | 5 60 | 0 57 | 7 30 | 0 63 | 8 11 | 2 15 | 27 60 |

lowest urine pH = 6 6 7 1 acid load test (6 g/sqm of body surface ammonium chloride given orally) urine pH after 2 hours was 6 8 after 4 hours 6 6 on concentrating test maximum urine concentration after 18 hours of water deprivation was 1 008 creatinine clearance 174 ml/min/1 73 sqm osmolar clearance 4 37 ml/min 1 73 sqm free water clearance +2 17 ml/min/1 73 sqm phosphorus clearance 27 ml/min/1 sqm potassium clearance 22 7 ml/min 1 73 sqm Urine alpha aminonitrogen was 98 mg/24 h and urine ammonium was 7 2 mEq/24 h A galactose loading test (1 g/kg body weight) showed a urinary elimination of 0 6 g in the first 4 hours after administration

X ray examinations of the skeleton (long bones) showed marked osteoporosis and rickets type growth cartilage (Fig 1) The bone age was normal (Greulich & Pyle atlas) Simple renal roentgenography revealed multiple calcifications with characteristic disposition at the pyramidal apices as small bunches (Fig 2) The renal cortex was spared An intravenous pyelogram showed in addition enlarged and loose calices (Fig 3) with hypotonic renal pelvis

COMMENT

The patient presented renal tubular disturbances renal tubular acidosis compromised concentrating function potassium and phosphorus urinary loss These marked renal troubles have produced an important growth retardation and signs of renal rickets No signs of glomerular impairment were present In spite of negative urinary data (insignificant bacteriuria repeated negative cultures absence of albuminuria normal leukocyturia) it is not possible to exclude a chronic pyelonephritis and the patient had had previously a positive culture for *E coli* in urine

A capital feature of the case is the presence of hypergammaglobulinaemia and recurrent purpura Hyperglobulinaemic tubular acidosis has been frequently reported in the recent literature It was observed in such conditions as multiple myeloma (17) lupoid hepatitis (12) active chronic hepatitis (15 16) hyperglobulinaemic purpura (2) idiopathic hyperglobulinaemia (22) Sjogren's syndrome (12 17) cryoglobulinaemia (19) and systemic



*Fig 1* X ray film of the knee Decreased bone density cortical atrophy and rickets type of growth cartilage are present

CASE REPORT

# RENAL TUBULAR ACIDOSIS AND HYPERGAMMAGLOBULINAEMIC PURPURA IN A 10 YEAR OLD GIRL WITH ROENTGENOGRAPHIC SIGNS SUGGESTING MEDULLARY SPONGE KIDNEY

M POPA and V STANESCU

*From the Institute of Endocrinology Bucharest Romania*

The association of hypergammaglobulinaemia with renal tubular acidosis has been described in multiple myelomatosis (18) and in a variety of conditions accompanied by hypergammaglobulinaemia (10 11 15 16 22) among which hypergammaglobulinaemic purpura (2) The association of a renal acidification defect with medullary sponge kidney was described by Levin (8) Morris *et al* (13) and Deck (3) In the present report a child presenting renal tubular acidosis hypergammaglobulinaemic purpura and roentgenographic signs suggesting medullary sponge kidney is described

## CASE REPORT

A 10 year old girl was admitted because of severe weakness bone pains petechiae on the lower extremities and retardation in physical growth The family history was irrelevant and in the past history several episodes of tonsillitis had occurred The disease was insidious in its onset and progressive About 4 years previously fatigue weakness decreased appetite and loss of weight were noted and became more and more intensive About 1 year previously diffuse bone pains appeared and the muscular weakness become severe so that walking was almost impossible In the last year in several afebrile periods petechiae were observed on the lower extremities Investigations performed in an outpatient service the same year showed increased erythrocyte sedimentation rate (1 hour 59 mm 2 hours 97 mm) and leukocytosis (10 900/ml) The diuresis was 1500–1800 ml/24 hours specific gravity of the urine was 1 007 A urine culture was positive for *E coli* but no germ or colony counts were performed The physical examination on admission in our department showed an underpoorly nourished girl (height 118 5 cm weight 1?2 kg) afebrile presenting severe muscular weakness and diffuse bone pain The patient appeared pale and on the malleolar region petechiae without pigmentation were noted No lymph nodes were palpable The chest examination was normal The liver was palpable at the right costal margin The spleen was not felt No masses were palpable in the renal regions Blood pressure was 100/60 mm Hg and pulse rate was 80–84 per minute The tourniquet sign was negative There were no other physical findings to note The neurological examination was negative No signs of neuromuscular hyperexcitability were found Polyuria was present (1600–1900 ml/24 hours) Specific gravity of the urine was 1 002–1 004 and gave a negative test for protein and sugar The sediment contained rare white cells and no red cells per high power field White cells excretion was 4240/minute and red cell excretion was 1280/minute (Addis method) Germ counting in urine was 1000/ml and repeated urine cultures were negative

The hematological and biochemical investigations were the following hemoglobin 13 9 g/100 ml erythrocyte sedimentation rate 88 mm at 1 hour 11? mm at 2 hours platelets count 146 000/ml serum sodium 135 6 mEq/l serum potassium 2 77 mEq/l serum chloride 112 mEq/l carbon dioxide combining power 14 9 mEq/l serum calcium 5 49 mEq/l serum inorganic phosphorus 1 9 mg/100 ml blood sugar 0 86 g/l serum cholesterol 1 50 g/l serum alkaline phosphatase 16 9 Bodansky units serum osmolality 250 mOsm/l plasma electric resistivity 72 Ω cm serum proteins 7 8–9 6 g/100 ml bleeding time 3 minutes clotting time 7 minutes The serum electrophoretic patterns are presented in Table 1 The immunoelectrophoretic assay revealed neither additional precipitation lines nor evident alterations in the size of the lines

The renal function tests performed were the fol

Table 1. *Serum electrophoresis at 1 month interval without treatment*

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## CASE REPORT

A 10 year old girl was admitted because of severe weakness bone pains petechiae on the lower extremities and retardation in physical growth The family history was irrelevant and in the past history several episodes of tonsillitis had occurred The disease was insidious in its onset and progressive About 4 years previously fatigue weakness decreased appetite and loss of weight were noted and became more and more intensive About 1 year previously diffuse bone pains appeared and the muscular weakness become severe so that walking was almost impossible In the last year in several afebrile periods petechiae were observed on the lower extremities Investigations performed in an outpatient service the same year showed increased erythrocyte sedimentation rate (1 hour 59 mm 2 hours 97 mm) and leukocytosis (10 900 ml) The diuresis was 1500–1800 ml/24 hours specific gravity of the urine was 1 007 A urine culture was positive for *E coli* but no germ or colony counts were performed The physical examination on admission in our department showed an mode[illegible] poorly nourished girl (height 118 5 cm we[illegible] kg) afebrile presenting severe muscular weakness diffuse bone pain The patient appeared pale and on the maleolar region petechiae without [illegible] were noted No lymph nodes were palpable The chest examination was normal The liver was palpable at the right costal margin The spleen was not [illegible] No masses were palpable in the renal region Blood pressure was 100/60 mm Hg and pulse rate was [illegible] 84 per minute The tourniquet sign was [illegible] There were no other physical findings to note The neurological examination was negative No signs of neuromuscular hyperexcitability were found Polyuria was present (1600–1900 ml/24 hours) Specific gravity of the urine was 1 002–1 004 and gave a negative [illegible] for protein and sugar The sediment contained [illegible] white cells and no red cells per high power field White cells excretion was 4240/minute and red cell excretion was 1280/minute (Addis method) Germ counting in urine was 1000/ml and repeated [illegible] cultures were negative

The hematological and biochemical investigations were the following hemoglobin 13 9 g/100 ml erythrocyte sedimentation rate 88 mm at 1 hour 11[illegible] mm at 2 hours platelets count 146 000/ml serum sodium 135 6 mEq/l serum potassium 2 77 mEq/l serum chloride 112 mEq/l carbon dioxide combining power 14 9 mEq/l serum calcium 5 49 mEq/l serum inorganic phosphorus 1 9 mg/100 ml blood sugar 0 86 g/l serum cholesterol 1 50 g/l serum alkaline phosphatase 16 9 Bodansky units serum osmolality 250 mOsm/l plasma electric resistivity 7[illegible] cm serum proteins 7 8–8 6 g/100 ml bleeding time 3 minutes clotting time 7 minutes The serum electrophoretic patterns are presented in Table 1 The immunoelectrophoretic assay revealed neither additional precipitation lines nor evident alterations in the size of the lines

The renal function tests performed were the fol

itary immunological abnormality with a heightened antibody response to a normal endogenous antigen producing a less obvious auto-immune disease. The action of filtered gammaglobulin on the tubules (5) or of an unknown substance acting at the same time as antigen and as nephrotoxin (10) as well as the impairment of kidney peritubular circulation by the increased blood viscosity (16) were also discussed.

As stated by Pyrah (15) the nephrocalcinosis with localizations of many small calcifications at the pyramidal apices and sparing of the cortex is characteristic for medullary sponge kidney and differentiates the latter from other nephrocalcinosis producing conditions. These typical calcifications were present in our case although the decisive urographic proof (medullary cystic like cavities) for medullary sponge kidney was absent.

Various degrees of tubular function involvement may be present in medullary sponge kidney: slight hyperchloremic acidosis (4), defective renal acidification without systemic acidosis (13), impaired ammoniuria (6, 8), defects in urine acidification, hydrogen and ammonium ion excretion (3). The more complete cases with systemic acidosis (3) are accompanied by pyelonephritis. In the study by Ekstrom *et al.* (4) inflammatory changes were present in all the medullary sponge kidneys examined histologically, although signs of clinical infection had been absent.

The clinical roentgenological entity of the medullary sponge kidney seems to be heterogeneous (13). Thus there were described some cases in successive generation (13), in siblings (1, 5) or associated with other congenital abnormalities (5, 13) among which Ehlers Danlos syndrome (9). In the last mentioned case the renal anatomical defect was associated with tubular acidosis without hyperglobulinaemia. In all these cases medullary sponge kidney represents probably a congenital abnormality of hereditary or developmental origin.

On the other hand, some cases of medullary sponge kidney might represent an anatomical deformity subsequent to other renal primary defect. The disease is discovered generally in adulthood (4, 13, 14) and rarely in children. Vermooten (21) suggests that uric acid crystals in the collecting tubules lead to stasis and dilatation during intra-uterine life. Deck (3) supposed that in some cases renal tubular acidosis is the primary defect. The consequent development of calculi would arise as a result of incomplete obstruction and dilatation of tubules. Finally the obstructive concretions might rupture into the renal pelvis leaving behind a cystic like cavity. The cases of Deck (3) with medullary sponge kidneys and renal tubular acidosis had a positive family history for tubular acidosis but immunoglobulin system of patients and their kindreds was not investigated.

In our case the primary defect might be the hypergammaglobulinaemia with subsequent tubular acidosis and nephrocalcinosis. One of the consequences of these renal alterations might be the medullary sponge kidney and it is possible that cystic like cavities would appear later at X-ray examination in accordance with the natural history of the disease. A renal developmental defect as a result of familial alterations in the immunoglobulin system influencing the developing kidneys might be considered too.

It seems reasonable to perform systematic urographic examination of patients with hyperglobulinaemic tubular acidosis as well as an examination of the immunoglobulins in the patients with medullary sponge kidneys, especially in the cases associated with tubular acidosis.

## SUMMARY

A 10-year-old girl presenting roentgenographic signs suggesting medullary sponge kidney, renal tubular acidosis and hypergammaglobulinaemic purpura is reported. The possible relationships between this conditions are discussed.



*Fig 2* Simple renal roentgenography Many calcifications bunched together as small groups at the pyramidal apices are observed

lupus erythematosus (20) The renal disturbance which is present in some cases of multiple myeloma is producing a *more* or less complete Fanconi syndrome with proximal tubular acidosis (10) This is probably due to the t bd damage caused by reabsorbtion of parap teins Harrison & Blamey (7) reported Fanconi syndrome in a patient with mo clonal abnormality of immunoglobulin he chain In the cases of hypergammaglobula mia the renal acidosis is of distal type (10) *these* cases hyperkaliuria, hypercalcuria wi out hypercalcaemia, pitressin resistant po uria nephrolithiasis, nephrocalcinosis a hyperphosphaturia are sometimes found

The relationship between hypergamma globulinaemia and renal tubular acidosis is unclear The biopsy specimens showed no monstrable immunoglobulin deposition in the glomeruli or the tubuli by immunofluoresc antibody studies (10 22) but a positive re tion for the C 3 component of serum co plement was observed in the glomeruli (? Prednisone therapy did not affect the respo to ammonium chloride loading (22) Wi *et al* (22) described cases of renal tub acidosis with immunoglobulin abnormalities their patients and in the kindreds of th patients without tubular acidosis They fo elevations of IgG and IgM in one family IgG in another one and in both familie serum anti gamma globulin factor against a genic site revealed by pepsin digestion of human IgG The authors suggested a b



*Fig 3* Intravenous pyelogram Enlarged and loose calices are observed together with multiple pyramidal calcifications The renal cortex is respected Hypotonia of the renal pelvis is also noted

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CASE REPORT

# ACCIDENTAL ASPIRATION OF TALC

*Report of a Case in a Two-Year Old Child*

JØRGEN SWANE LUND and MARGIT FELDT RASMUSSEN

*From the Departments of Paediatrics and Anaesthesia Gentofte Hospital Copenhagen Denmark*

There have been surprisingly few reports on the serious risk of accidental inhalation of talc containing dusting powder in infants Only four cases have been published three of them fatal (1 3 4 5)

This is the report of another such case which was successfully treated in close collaboration between the paediatric and anaesthetic departments

## CASE REPORT

During the care at home of a 24 months old girl the bottom of a baby powder container broke open and probably about 50 grams of the powder emptied directly over the face of the child

On admission, shortly after the patient was conscious, but limp pale slightly cyanotic and showing signs of respiratory distress with a respiratory rate of 60–70 min and intercostal retractions She vomited several times

X ray of the chest showed small particles of talc in the bronchi (and in the gastric fundus) and small hilar and perihilar infiltrations

Treatment was begun immediately with Actocortin® 100 mg i.m. followed by Prednisone 5 mg 4 times daily antibiotics in the form of sodium penicillin 1,000 000 units twice daily and the patient was placed in an oxygen Alevaire® tent Inhalation of Alupent® was tried without visible effect

During the first 40 hours the condition remained almost stationary the respiratory rate being about 50 min pulse rate about 130 min and the temperature slightly rising About 45 hours after admission the temperature rose to 40°C, and the pulse rate to 150–160 min The patient became increasingly restless with manifest respiratory insufficiency (respiratory rate 50–60 min interrupted by periods of apnoea) $P_{CO_2}$ increased to 48 mm Hg while the pH dropped to 7 10 (Table 1)

Oro tracheal intubation was done and intermittent positive pressure breathing (I P P B ) started (a peak pressure of 40–50 cm of water was needed to maintain a sufficient alveolar ventilation) A small pneumothorax on the left side developed and demanded drainage Artificial ventilation had to be administered continuously for 24 hours intermittently for 12 hours whereafter the patient could breathe spontaneously and was extubated. During the period of intubation sedation was required with Pethidine hydrochloride in 5 mg doses and Promethazine in 4 mg doses

X ray control of the lungs showed a development from the initial small perihilar changes to larger partly confluent infiltrations (coinciding with the clinical exacerbation) and thereafter a gradual regression to normal

The patient was discharged in good condition on the 12th day Her electroencephalogram was normal at discharge

## DISCUSSION

The powder in question consists of 90 talc 3 boric acid 7 zinc oxide and about 0 02 lanolin Talc is a silicate ($Mg_3Si_4O_{10}(OH)_2$) which produces histiocytosis and tissue granuloma formation (2)

The autopsy reports from three of the four previous cases showed identical findings viz. bronchitis and peribronchitis pulmonary oedema, and extensive segmental atelectases alternating with compensatory emphysema in both lungs (1 4 5) In two cases dilatation of the right heart was found

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CASP REPORT

# HYDRANENCEPHALY

## *A Case Report with Autopsy Findings in a 7 year old Girl*

JULIUS HOFFMAN and LEOPOLD LISS[1]

*From the Department of Pediatrics Howard University College of Medicine Washington D C., U.S.A*

Hydranencephaly is a congenital anomaly in which the cerebral hemispheres are replaced by membranous sacs filled with cerebrospinal fluid The meninges are intact and the cranium is usually of normal size and proportions Most of the cerebral tissue is absent and what may remain usually is found in the basal areas of the occipital and temporal lobes Though the basal ganglia and the rostral portions of the midbrain are intact, they too may be absent or defective Except for agenesis of the long descending tracts the remaining nervous tissue generally is normal

Though the exact incidence is unknown the mounting awareness of this disease and particularly the more frequent use of transillumination as a part of the pediatric neurologic examination has resulted in increasing numbers of reports of infants diagnosed early in life

With the vast amount of brain substance lacking in this disease it would be quite expected that this condition is usually discovered in infants who have died at birth or within a few weeks after birth for it seems incompatible with prolonged life (1) Indeed after a review of the literature it is believed that the 7½-year-old patient discussed here is the oldest known patient with hydranencephaly

## CASE PRESENTATION AND HISTORY

D K W a female aged 7 years 6 months was admitted for the first time to the hospital for treatment of fever and pneumonia The past history revealed that after an eight month gestation, complicated by a mild maternal renal infection at 5 / months, the patient was born by Caesarean section because of a cephalo pelvic disproportion The birth weight was 3869 g A previous pregnancy was accompanied by frequent fainting episodes throughout gestation but resulted in the normal spontaneous delivery of a healthy child A subsequent pregnancy terminated in a six month spontaneous abortion of unknown etiology No abnormality was detected in the fetus At birth, there was some mild difficulty in resuscitation otherwise she appeared normal At 3 weeks of age the patient was first noted to be jaundiced and was hospitalized for 3 to 4 months While in the hospital she remained in the prone position and developed stiff muscles with the upper extremity flexed" at the elbow Head control and other expected developmental milestones were observed Prior to admission to another hospital at 2 / years, she had learned to drink from a cup when propped in the sitting position A neurological examination at that time was normal except for a left lateral nystagmus and internal squint. Transillumination was not done

The child was cared for at home most of her life with surprisingly few illnesses She did have rubella, rubeola, and mumps—all without sequelae She was placed in nursing homes for family reasons and with each admission a pediatric examination was done without any new findings other than those related to cerebral palsy"

Two days before admission to the hospital, she developed a rapidly progressing anorexia and fever and on the basis of a recent outbreak of staphylococ

Presented at the American Academy of Pediatrics Meeting, Washington D C October 26 1967

[1] Presently Professor of Pathology Ohio State University Columbus, Ohio

Table I *Capillary blood gases during treatment of a case of talc aspiration*

| | 6 4 67 2nd hospital day | 7 4 67 6 a m * | — 9 a m | — 11 a m | 8 4 67 ** | 9 4 67 | 10 4 67 |
|---|---|---|---|---|---|---|---|
| Pco (mm Hg) | 33 | 48 | 47 | 40 | 37 | 34 | 3? |
| pH | 7 32 | 7 10 | 7 26 | 7 44 | 7 35 | 7 49 | 7 40 |
| Base excess (mEq per litre) | −8 4 | −12 5 | −5 2 | −2 2 | −4 6 | +3 | +12 |

* Start of I P P B + bicarb 70 mEq ** End of I P P B

Microscopic examination showed the most marked changes in the bronchial tree in the form of epithelial desquamation and basophilic masses obliterating the bronchial lumina partially or completely and at times forming alveolar casts (5) These masses consisted of leukocytes epithelial fragments, and crystalline particles embedded in a homogeneous matrix There was pronounced leukocytic infiltration of the affected tissues indicating the inflammatory nature of the processes

A characteristic clinical feature of these cases as well as of ours is cyanosis, tachypnoe (up to 90/min) with intercostal retractions and tachycardia (160–190/min)

In all the four reported cases the treatment consisted of O and antibiotics, but in addition two had digitalis, one a diuretic one lobeline and coramine® and one morphine The only reported survivor received in addition steroids when the condition deteriorated (the respiratory rate rose to 80–90/min there was cyanosis but neither auscultatory nor radiographic changes) The improvement seemed to be related to the anti inflammatory effect of the steroid

In our case the condition deteriorated in spite of immediate steroid therapy Auscultation revealed fine crepitation over both lungs and X rays showed signs of bronchitis and peribronchitis with atelectases the same changes which were described in the three fatal cases The patient developed a combined respiratory and metabolic (hypoxic) acidosis which was treated effectively with artificial ventilation and administration of bicarbonate The respiratory failure occurred relatively late compared to the previously reported cases This may be attributed to the steroid therapy

The artificial ventilation is the logical treatment of manifest respiratory failure but in retrospect it is realized that intermittent assisted ventilation by bag and mask instituted at an earlier stage (before acidosis became marked) might have prevented the respiratory failure and made intubation unnecessary

## SUMMARY

A case of talc inhalation in a two year old girl with alarming clinical symptoms was successfully treated with steroids and artificial ventilation In only one of four previously published cases the child survived

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## A Case Report with Autopsy Findings in a 7 year-old Girl

JULIUS HOFFMAN and LEOPOLD LISS[2]

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[Hy]dranencephaly is a congenital anomaly in [wh]ich the cerebral hemispheres are replaced [by] membranous sacs filled with cerebrospinal [flu]id The meninges are intact and the cranium [is] usually of normal size and proportions Most [of] the cerebral tissue is absent and what may [re]main usually is found in the basal areas of [th]e occipital and temporal lobes Though the [ba]sal ganglia and the central portions of the [mi]dbrain are intact they too may be absent [o]r defective Except for agenesis of the long [d]escending tracts the remaining nervous tissue generally is normal

Though the exact incidence is unknown the mounting awareness of this disease and particularly the more frequent use of transillumination as a part of the pediatric neurologic examination has resulted in increasing numbers of reports of infants diagnosed early in life

With the vast amount of brain substance lacking in this disease it would be quite expected that this condition is usually discovered in infants who have died at birth or within a few weeks after birth for it seems incompatible with prolonged life (1) Indeed, after a review of the literature it is believed that the 7 1/2-year-old patient discussed here is the oldest known patient with hydranencephaly

[1] Presented at the American Academy of Pediatrics Meeting Washington D.C. October 26 1967

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## CASE PRESENTATION AND HISTORY

D. A. W., a female aged 7 years 6 months was admitted for the first time to the hospital for treatment of fever and pneumonia. The past history revealed that after an eight month gestation complicated by a mild maternal renal infection at 5 1/2 months, the patient was born by Caesarean section because of a cephalopelvic disproportion The birth weight was 3840 g. A previous pregnancy was accompanied by frequent fainting episodes throughout gestation but resulted in the normal spontaneous delivery of a healthy child A subsequent pregnancy terminated as a six month spontaneous abortion of unknown etiology No abnormality was detected in the fetus At birth, there was some mild difficulty in resuscitation otherwise she appeared normal. At 5 weeks of age the patient was first noted to be mongoloid and was hospitalized for 3 to 4 months While in the hospital she remained in the prone position and developed stiff muscles, with the upper extremity "flexed" at the elbow Head control and other expected developmental milestones were observed Prior to admission to another hospital at 2 1/2 years she had learned to drink from a cup when propped in the sitting position A neurological examination at that time was normal except for a left lateral nystagmus and internal squint. Transillumination was not done

The child was cared for at home most of her life with surprisingly few illnesses She did have rubella, rubeola, and mumps—all without sequelae She was placed in nursing homes for family reasons and with each admission a pediatric examination was done without any new findings other than those related to cerebral palsy"

Two days before admission to the hospital, she developed a rapidly progressing anorexia and fever and on the basis of a recent outbreak of staphylococ

Table 1 *Capillary blood gases during treatment of a case of talc aspiration*

| | 6 4 67 2nd hospital day | 7 4 67 6 a m * | — 9 a m | — 11 a m | 8 4 67 * | 9 4 67 | 10 4 |
|---|---|---|---|---|---|---|---|
| Pco (mm Hg) | 33 | 48 | 47 | 40 | 37 | 34 | 3? |
| pH | 7 32 | 7 10 | 7 26 | 7 44 | 7 35 | 7 49 | 7 4? |
| Base excess (mEq per litre) | −8 4 | −12 5 | −5 2 | −2 2 | −4 6 | +3 | +1? |

Start of I P P B + bicarb 70 mEq   * End of I P P B

Microscopic examination showed the most marked changes in the bronchial tree in the form of epithelial desquamation and basophilic masses obliterating the bronchial lumina partially or completely and at times forming alveolar casts (5) These masses consisted of leukocytes epithelial fragments, and crystalline particles embedded in a homogeneous matrix There was pronounced leukocytic infiltration of the affected tissues indicating the inflammatory nature of the processes

A characteristic clinical feature of these cases as well as of ours is cyanosis, tachypnoe (up to 90/min) with intercostal retractions and tachycardia (160–190/min)

In all the four reported cases the treatment consisted of O and antibiotics but in addition two had digitalis one a diuretic one lobeline and coramine® and one morphine The only reported survivor received in addition steroids when the condition deteriorated (the respiratory rate rose to 80–90/min there was cyanosis but neither auscultatory nor radiographic changes) The improvement seemed to be related to the anti inflammatory effect of the steroid

In our case the condition deteriorated in spite of immediate steroid therapy Auscultation revealed fine crepitation over both lungs, and X rays showed signs of bronchitis and peribronchitis with atelectases the same changes which were described in the three fatal cases The patient developed a combined respiratory and metabolic (hypoxic) acidosis which was treated effectively with artificial ventilation and administration of bicarbonate

The respiratory failure occurred relatively la[te] compared to the previously reported case[s] This may be attributed to the steroid therap[y]

The artificial ventilation is the logical treat ment of manifest respiratory failure but i[n] retrospect it is realized that intermittent a[s] sisted ventilation, by bag and mask instituted at an earlier stage (before acidosis became marked) might have prevented the respiratory failure and made intubation unnecessary

## SUMMARY

A case of talc inhalation in a two year old girl with alarming clinical symptoms was successfully treated with steroids and artificial ventilation In only one of four previously published cases the child survived

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CASE REPORT

# HYDRANENCEPHALY

## *A Case Report with Autopsy Findings in a 7 year old Girl*

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Hydranencephaly is a congenital anomaly in which the cerebral hemispheres are replaced by membranous sacs filled with cerebrospinal fluid The meninges are intact and the cranium is usually of normal size and proportions Most of the cerebral tissue is absent and what may remain usually is found in the basal areas of the occipital and temporal lobes Though the basal ganglia and the rostral portions of the midbrain are intact they too may be absent or defective Except for agenesis of the long descending tracts the remaining nervous tissue generally is normal

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*Fig 1* Appearance of contents of skull on removal of calvarium

cal pneumonia at the nursing home it was felt that the child had probably contracted a pneumonia

Detailed inquiry revealed no evidence of familial neurological disease

On physical examination she was seen to be a moribund gasping cyanotic female with fever and respiratory distress She was cachectic and had many contractures The head was normal in size and slightly dolicocephalic The pupils were mydriatic and equally reactive to light directly and consensually The eye movements were rapid and incoordinated Ophthalmoscopic examination revealed no abnormalities though she failed to follow a moving light and did not fix her gaze The deep tendon reflexes were equal bilaterally and somewhat hyperactive No ankle clonus or Babinski toe reflexes were obtained

The admission hemogram showed 14 000 WBCs with a lymphocytosis Nasopharyngeal and throat cultures were normal Culture of the eye drainage produced Hemophilus influenzae Chest X rays revealed marked scoliosis but no gross pneumonic consolidation The admission diagnoses were (1) bronchopneumonia (2) spastic cerebral palsy and (3) mental deficiency

After antibiotic and bacteriostatic therapy she became afebrile However after two unexplained apneic episodes within 7 hours she was unable to be resuscitated and she died at the age of 7 years 6 months

On postmortem examination it was observed that the body was poorly developed and poorly nourished There appeared to be contractures at most joints The heart adrenals thymus and kidneys weighed from 20–30 % below the expected norm However there were no apparent morphological abnormalities of these organs

The tissues of the scalp were normal and the fon

tanelles and sutures were closed There was no abnormality noted in the bony structures externally or internally After the dura mater was opened watery clear spinal fluid ran out of the skull The falx cerebri was small, but the tentorium cerebelli appeared normal All of the dural sinuses were patent and of small caliber The superior sagittal sinus measured approximately 5 cm in length to the confluence of the sinuses The great cerebral vein was patent

The brain weighed 270 g and revealed the following gross anatomical features the cerebral cortex was absent except for occipital, insular hippocampal and temporal remnants Except for a reduction in the size of the cerebral peduncles and an absence of medullary pyramids the brain stem and spinal cord appeared normal and the cerebellum was characterized by well developed folia hemispheres and a vermis The gross appearance of the forebrain was two bilateral bulb like masses with small lateral projections from each mass. The circle of Willis was apparently normal but the middle and anterior cerebral arteries were of extremely small caliber and patent. All cranial nerves were present and reduced in size The pituitary gland appeared to be normal

## DISCUSSION

The etiology of hydranencephaly is still very controversial The currently accepted hypothesis is that a circulatory disturbance occurring in fetal life is the responsible pathogenic factor It has been postulated that this is due to compression of the fetal carotid arteries by the umbilical cord or amniotic adhesions

It would appear that the disorder represents a failure of development of the cerebral mantle prior to the second month of fetal life

It is particularly significant that the patient we are discussing was similar to the many others reported in that no abnormal neurological signs at birth or even up to three weeks of age were present to suggest that she was not entirely normal and that she was almost completely without cerebral hemispheres

If the baby is not born dead the clinical pattern is usually one of relatively unremarkable prenatal natal and neonatal course At variable intervals afterward adaptive and neuromuscular development retardation becomes increasingly apparent Occasionally a mild enlargement of the head may be noted Gradually feeding difficulties incoordinated eye movements nystagmus strabismus and signs of hyperirritability may occur The optic discs appear small and pale though visual blinking reflex is preserved and the pupils may react well to light directly and consensually though generally visual perception is absent Percussion of the skull yields a tympanitic or cracked pot sound as may be expected in the baby with open fontanelles Seizure activity if present, can take any form including opisthotonus tremors twitching movements rigidity and even Jacksonian and grand mal[1] Failure of normal mental and adaptive development become obvious and the child is usually considered and treated as mentally retarded and/or cerebral palsied[2]

Comparatively simple procedures are available to confirm the presence of hydranencephaly Transillumination of the skull should be a routine part of the pediatric neurological examination Not only in hydranencephaly but also in subdural hematomas subdural effusions subdural hygromas etc transillumination is also of diagnostic aid The technique involves a standard two-cell flashlight with a foam rubber cuff around the source of light The foam rubber cuff allows molding to the curvatures of the skull and in addition prevents light reflection from the rim of the flash light. The subject is examined in a totally darkened room In the case of a hydranencephalic infant the entire cranium will glow with an orange red light like an illuminated Japanese lantern

The present report and study clearly demonstrates several important points

1 The importance of transillumination as a diagnostic aid in the neurological examination of infants and children

2 The apparent normal appearance in early stages of development of patients with hydranencephaly

3 The relative lack of clinical functional expressivity of the frontal and parietal lobes in the overall early development particularly as elicited by current pediatric neurological examination techniques

4 The support of the hypothesis that hyd

ranencephaly is pathogenetically related to diminished carotid artery patency

5 Hydranencephaly is not incompatible with life beyond infancy

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CASE REPORT

# INCREASING METABOLIC ACIDOSIS FOLLOWING FRUCTOSE INFUSION IN TWO CHILDREN

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Solutions with a high content of fructose are widely used as a source of energy for parenteral nutrition in postoperative and posttraumatic care as well as in the treatment of conditions involving hepatic insufficiency and metabolic acidosis Fructose is eliminated more rapidly than glucose from the bloodstream and its metabolism has been considered to be less dependent on insulin (6 8) Fructose has consequently come to be used in diabetic acidosis (6) It has long been recognized (7)—and new studies have recently confirmed (1)—that part of the fructose in the liver is metabolized into lactic acid It has however been considered that the increase in blood lactate after fructose infusion is relatively slight and of no practical clinical importance (5 6 11) It has been shown that the administration of fructose also in newborn infants result in formation of lactic acid (3) It is of importance to be aware of this fact as small children are very sensitive to alterations of pH and serum electrolytes

This paper concerns two young children with metabolic acidosis who were treated with fructose infusion One child died but the other survived probably because the infusion was interrupted in time In view of these cases and the results of metabolic studies particularly those by Bergström *et al* (2) we recommend a reappraisal of the suitability of fructose as an agent for parenteral nutrition

## CLINICAL DATA AND RESULTS

*Case I*

A 1 year-old boy was admitted on Dec 23 1966 to the Clinic for Infectious Diseases Danderyds Hospital Danderyd 3 Sweden, for gastroenteritis His grandmother had acquired diabetes mellitus as an elderly woman but no other diabetic relatives were known. Pregnancy and delivery normal Birth weight 5210 g Normal development Healthy before the present illness He started vomiting and had diarrhoea on Dec 19 his temperature rose to 39 C on Dec 20 The fever was lower on the next two days but the vomiting and diarrhoea became more frequent The boy was admitted to hospital on the third day of his illness

We saw him first at 1 a m on Dec 23 and found him moderately dehydrated with an acidosis (Fig 1) His general condition however was only slightly affected and no other abnormalities were observed at the physical examination Laboratory tests showed a normal haemoglobin value (12 9 g/100 ml) a white cell count of 8400 with a slight increase in neutrophils Micro-ESR was 10 mm/hour hematocrit 39 ml/100 ml potassium 4 1 mEq/l sodium 135 mEq/l chloride 132 mEq/l urea N 22 mg/100 ml bicarbonate 13 2 mEq/l and base deficit 12 0 mEq/l Urine was normal with no protein or sugar Chest X ray and ECG were normal Lumbar puncture showed a normal liquor When the results of cultures from the nose throat and stools became available later only bacteria normally found in such specimens had been isolated

Continuous intravenous infusion was started at 2 p m on Dec 23 The solution contained 20 % fructose and it was planned to add sodium chloride and bicarbonate when the serum electrolytes had been estimated During the next two hours the boy seemed fairly well Urine was excreted in a satisfactory amount He vomited once and passed one loose stool. At about 5 p m however his breathing steadily deepened the blood pressure fell the skin became

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intravenously as well as intermittently during the next few hours (Fig 2) A 5 5 % glucose solution with potassium 20 mEq/l, was given continuously The acidosis as well as the serum potassium were corrected in the course of the day (Fig 2) In the urine ketone bodies were demonstrated even during Jan 1 and 13 During the therapy on Jan 12th the breathing became normal and the signs of shock disappeared but the child did not become fully conscious until the evening

DISCUSSION

Both children had a metabolic acidosis before the start of the fructose infusion Even so blood bicarbonate decreased markedly some time after the start of the fructose infusion (Figs 1 and 2) in spite of the administration of large doses of bicarbonate at the same time as the infusion When the fructose infusion was reduced or withdrawn pH and bicarbonate increased In view of the reports of Bergstrom & Hultman (1 2) it is suggested that the increasing acidosis in our children was due to formation of lactic acid from the fructose The above mentioned authors have shown that the administration of fructose in normal subjects results in formation of lactic acid In patients with tissue hypoxia due to circulatory disturbances this lactate formation will be still more pronounced Fructose has even been considered to be more advantageous than glucose in severe acidosis (6) and it has consequently been recommended that fructose should be administered to patients with liver damage and circulatory failure and to those with ketonemia (6 9)

The following discussion of the carbohydrate metabolism is pertinent to an understanding of why fructose gives a higher lactate formation than glucose Glucose is converted at cell level into glucose 6-phosphate which is then turned into fructose 6-phosphate This then enters the glycolytic pathway where it is metabolized into pyruvic acid energy is supplied to the body when the pyruvic acid is metabolized in the citric acid cycle If there is no energy requirement, the glucose 6-phosphate is instead converted into glucose 1 phosphate which is then converted into glycogen It might be supposed that fructose—by being converted with the aid of hexokinase into fructose 6 phosphate—would give the same metabolic result as glucose It has been found however that in the liver fructose is chiefly converted into fructose 1 phosphate (with the aid of fructokinase) because the activity of fructokinase is greater than that of hexokinase in the liver (12) In the presence of aldolase fructose 1 phosphate is split into dihydroxyacetone phosphate and glyceraldehyde (4) Dihydroxyacetone phosphate can be converted into glyceraldehyde 3 phosphate (with the aid of triosisomerase) which can then be metabolized into fructose 6 phosphate and glycogen Glyceraldehyde on the other hand is converted via d glycerate into glyceraldehyde 2 phosphate which is then metabolized into pyruvate (10) This means that the supply of pyruvate may exceed the current energy requirement in which case part of the surplus will be converted into lactate The formation of lactate will be considerable especially under conditions of hypoxia

One of our patients died The autopsy showed a pronounced enteritis but it seems more likely to us that the death was a result of irreversible cell injury due to the extreme acidosis (pH 6 6) during fructose infusion rather than to the infection itself In view of these cases and the results of metabolic studies particularly those by Bergstrom *et al* (1 2) we recommend a reappraisal of the suitability of fructose as an agent for parenteral nutrition We consider that fructose infusions should be avoided in the following situations

1. Latent or manifest shock
2. Fluid imbalance with metabolic acidosis (particularly in children)
3. In connexion with surgery when correction of fluid and electrolytes is more important than calory supply
4. Intoxication with varying degrees of metabolic acidosis which may be due either to unstable blood pressure and hence a poor peripheral circulation or to the promotion of acidosis by the toxic agent

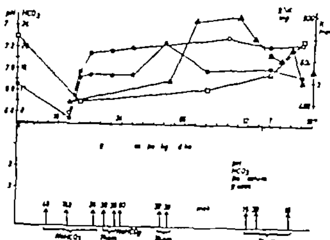


*Fig 1* Blood and plasma values of pH HCO potassium and glucose in a 1 year old boy with metabolic acidosis which increased during treatment with fructose infusion The boy died Fructose infusion (g/kg BW/hour) is indicated on the figure

cold and pale the pulse was rapid and he lost consciousness The boy obviously needed extensive antishock treatment He was intubated ventilation being subsequently taken over by an Engstrom respirator Blood albumin and fluid were given (20% fructose 10 fructose glucose) with electrolyte solutions (a large amount of hypertonic bicarbonate solution and tris buffer (Tham®) This therapy resulted in correction (not complete) of the acidosis and restoration of the electrolyte balance particularly the serum potassium (Fig 1) The systolic blood pressure rose to about 90–100 mm Hg Urinary excretion remained satisfactory A tendency to convulsions was treated with small doses of curare In the morning of Dec 24 the boy's condition seemed to have improved blood pressure had remained on a normal level for 9 hours the skin was dry and warm output of urine was quite good On the other hand there was still an acidosis to correct blood glucose was high in spite of high i v doses of insulin and the boy was not fully conscious The situation remained unchanged until about 11 a m when blood pressure slowly began to fall the acidosis again became more pronounced and at 4 p m there was cardiac arrest External heart massage caused the heart to start working again but the boy died at 11 p m

A complete autopsy including microscopic examination of all organs showed a pronounced enteritis and a small brain tumor in the region of the third ventricle This tumor was not considered to have had any influence on the disease Blood from the body contained no CF antibodies against herpes simplex virus adenovirus or mumps virus Nor could any cytopathogenic agent be isolated from the brain

*Case 2*

A 2 year old girl was admitted on Jan 11 1967 to the same clinic The girl's development was normal and she had been healthy before the present illness She had fallen ill on Jan 5 in a herpetic stomatitis with fever During six days of illness her temperature had gradually fallen to normal but she mostly refused food and took but small amounts of fluid On Jan 10 the mother gave her for the first time acetylsalicylic acid (250 mg × 4) No drugs were administered after that On the morning of Jan 11 the girl began to vomit and completely refused all fluid and food She was accordingly admitted to hospital late that evening

When we first saw the girl she was slightly exhausted but had no fever She smelled of acetone Breathing was normal Slight clinical signs of dehydration She had an extensive stomatogingivitis with numerous ulcers in the oral cavity Otherwise the physical examination showed normal results The urine contained ketone bodies Preliminary diagnosis primary herpetic stomatitis with slight dehydration At 2 a m it was obvious that a continuous intravenous infusion was essential and 500 ml of a Ringer solution containing 5.5% glucose was administered between 2 and 4 a m at which time the blood tests become available Hematocrit was 36 ml/100 ml sodium 138 mEq/l chloride 112 mEq/l potassium 6.2 mEq/l and bicarbonate 12.1 mEq/l The child had ceased to vomit and the excretion of urine seemed to be satisfactory To correct the acidosis and supply a high calory fluid 500 ml 20% fructose solution was given with 36 mEq/l $NaHCO_3$ The child slept for the next four hours and the situation seemed to be under control Between 8 and 8.30 a m however her breathing steadily deepened the skin turned a pale grey and the hands and feet became cold The child lost consciousness and it was obvious that the acidosis had suddenly become more pronounced in spite of the adequate fluid therapy The child was in shock

Remembering case 1 a fortnight earlier it seemed that the only factor common to both was the fructose solution The infusion of fructose solution was stopped Hypertonic $NaHCO_3$ was given immediately

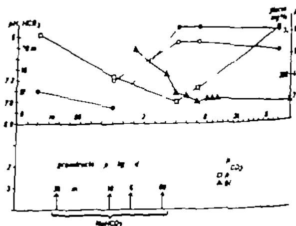


*Fig 2* Blood and plasma values of pH HCO potassium and glucose in a 2 year-old girl with metabolic acidosis which increased during treatment with *fructose infusion The girl survived* Fructose infusion (g/kg BW/hour) is indicated on the figure

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# PROCEEDINGS OF PEDIATRIC SOCIETIES

## EUROPEAN SOCIETY FOR PEDIATRIC GASTROENTEROLOGY

Meeting in Paris October 4–5 1968

B Hadorn J Presser (Berne) & M Messer (Melbourne) *Isolation of an activator of pancreatic trypsinogen from human duodenal contents*

A peptidase (enteropeptidase enterokinase) which is able to activate pancreatic trypsinogen has been isolated and purified from human duodenal juice It is a large molecular weight glycoprotein and is remarkably stable against the action of other proteolytic enzymes Upon DEAE-cellulose chromatography the purified enzyme splits up into two components which are still enzymatically active but have a smaller molecular weight than the crude enzyme Partial proteolysis not affecting the active center of the enzyme is thought to be responsible for this phenomenon The enzyme is inhibited by diisopropylphosphorofluoridate (DFP) and by a number of trypsin inhibitors suggesting that its active center resembles that of trypsin Purified entero-peptidase however does not attack synthetic trypsin substrates such as p Tosyl L arginine methylester (TAME)

A method suitable for the determination of enteropeptidase activity in human duodenal and ileal mucosa has been developed The conditions of the assay system are such that the activation of trypsinogen by enteropeptidase follows zero order kinetics

T Lindberg (Malmo) *Studies on intestinal dipeptidases*

Dipeptide splitting activity in the human intestinal mucosa has been studied by a spectrophotometric assay method The following ten dipeptides were used as substrates glycyl L leucine glycyl L valine glycylglycine L alanyl L glutamic acid L valyl L glutamic acid L glutamyl L valine L alanyl L proline L valyl L proline L glutamyl L proline and glycyl L glutamine

Developmental studies of five dipeptidase activities revealed that all were fully developed at 11 weeks of fertilization age i e in the smallest fetus studied

Distribution studies showed that in adults the stomach proximal duodenum and large intestine contain low dipeptidase activities while high activities were found in distal duodenum and jejunum ileum

Very low activities were found in intestinal juice

The activity against the ten different dipeptides in small intestinal biopsy specimens from about 200 patients (children and adults) with various gastro-intestinal disorders has been studied The various activities were related to the morphology of the mucosa in each case It was found that the more the mucosa was abnormal the more were the activities decreased Thus there is a clear correlation between the structure of the mucosa and the dipeptidase activity

However some patients had low activities against some dipeptides in spite of normal structure of the mucosa The significance of this finding is at present difficult to explain

Twenty patients (10 children and 10 adults) had gluten induced enteropathy All of these had significantly low dipeptidase activities but

## SUMMARY

Two children with metabolic acidosis received fructose infusion in accordance with the standard treatment at the time During the infusion both pH and bicarbonate decreased in spite of the administration of large doses of bicarbonate When the fructose infusion was reduced or withdrawn, pH and bicarbonate increased However the extreme acidosis (pH 6 6) during fructose infusion in one of the children had probably caused irreversible cell injuries which might have been the main reason for the death in this patient The other child survived probably because the fructose infusion was interrupted in time We accordingly recommend that fructose infusions should not be given to patients in whom there is a risk of acidosis and tissue hypoxia

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Developmental studies of five dipeptidase activities revealed that all were fully developed at 11 weeks of fertilization age i e in the smallest fetus studied

Distribution studies showed that in adults the stomach proximal duodenum and large intestine contain low dipeptidase activities while high activities were found in distal duodenum and jejunum ileum

Very low activities were found in intestinal juice

The activity against the ten different dipeptides in small intestinal biopsy specimens from about 200 patients (children and adults) with various gastro intestinal disorders has been studied The various activities were related to the morphology of the mucosa in each case It was found that the more the mucosa was abnormal the more were the activities decreased Thus there is a clear correlation between the structure of the mucosa and the dipeptidase activity

However some patients had low activities against some dipeptides in spite of normal structure of the mucosa The significance of this finding is at present difficult to explain

Twenty patients (10 children and 10 adults) had gluten induced enteropathy All of these had significantly low dipeptidase activities but

a general increase in the activities to almost the same levels as those of the controls occurred after treatment with gluten free diet

E Eggermont (Louvain) *The biochemical lesion of sucrose intolerance*

Kinetic studies with mixed substrates indicate that the small intestine of man forms glucose from maltose isomaltose sucrose and glycogen through the action of three enzymes Each of the enzymes hydrolyses maltose and one of the other substrates Therefore they are called maltase isomaltase maltase sucrase and γ amylase These α glucosidases are localised in the brush border membrane and can be solubilised with the use of the neutral detergent *Triton X 100* When the solubilised enzymes sediment in a density gradient they are recovered together in the fraction with a sedimentation coefficient of 10 S For most proteins this sedimentation coefficient would correspond to an approximate molecular weight of 210 000 However various experimental conditions such as mild exposure to heat (45°) ionic environment and solubilisation with papain disintegrate the 10 S peak and induce the appearance of both a faster (13 S) and a slower (6–7 S) component The ratios 13 10 6 of the sedimentation coefficients suggest that the molecular weights of the various components could be in the ratios 3 2 1 Thus if we translate sedimentation coefficients into molecular weights assuming that neither the specific volume nor the shape of the molecules are changed we would conclude that the native 10 S structure is a dimer (or a duplex if the subunits are not identical) and that the changes in sedimentation velocity arise from the dissociation of the dimer in monomers (6 S) which partly reassociate into trimers (13 S)

Hereditary sucrose intolerance is characterised by a multiple enzymic lesion affecting not only the maltase sucrase but also the maltase isomaltase and the γ amylase While the maltase sucrase is nearly absent, the maltase isomaltase and γ amylase are reduced to abo 10 and 35 of the normal levels respectively Furthermore, the genetic lesion results in qualitative changes of the sedimentation pattern of the residual enzymes the usual 10 S peak is replaced by two novel components with a sedimentation coefficient of 12 6 and 6 2 S respectively

As the polyenzymic abnormalities of sucrose intolerance are both qualitative and quantitative and as they are reminiscent of experimentally induced alterations of the normal enzymes they are best explained by a single mutation of a structural gene Up to now the incomplete knowledge of the normal structural organisation of the maltase isomaltase the maltase sucrase and the γ amylase prevent further determination of the affected polypeptide

G W Meeuwisse (Lund) *Experiences with a hydraulic multiple biopsy instrument for peroral intestinal biopsies in children*

The hydraulic intestinal biopsy instrument was constructed at the Lund University Hospital and it has now been in regular use during 2½ years The capsule made of stainless steel measures 4 × 12 millimeters and has a pair of side holes of 1 7 millimeters Suction pressure and the flush of water delivering the specimen (with the capsule remaining in situ) is mediated by compressed air or CO A pair of plastic tubes of 1 5 meters length connects the capsule with the suction and pressure unit

The author s experience comprises 93 intestinal biopsies on 64 individuals Most of them were done on children The smallest child weighed 2 2 kg when biopsies were taken from the jejunum at 2½ months of age Two children were younger than 2 months In 16 children the instrument was used more than once

An average of 8 specimens (weight 2–10 mg each) were taken per seance A suction of −0 atm was found suitable to aspirate mucosa into the capsule The specimens contain mu

osa muscularis mucosa and a little of the submucosa. Mucosal specimens from the colon and/or terminal ileum were obtained in 5 patients (youngest 7 years) by transintestinal intubation. The maximum number of biopsies taken in one such patient was 20.

Occult blood loss occurred in the majority of patients, but melaena or significant fall of Hb concentration did never occur. One severe complication occurred in the 30th patient. On him 12 biopsies were taken near each other in the distal duodenum. This 6 months old boy who was found to have a normal mucosa had to be operated the day after biopsy because of a perforation at the biopsy site. He fortunately recovered after operation. Since then the capsule is always pulled nasally 1–2 centimeters between each biopsy. With the capsule at levels beyond the jejunum the proximal part of the tube is displaced at least 5 centimeters between the biopsies.

Malfunction of the capsule (in 6 patients only one and at 4 first intubations no specimen was obtained) was found to depend on wear. With new capsules of hardened steel the need for frequent service by the instrument maker will probably be reduced, which seems to be necessary before the apparatus can be made available also for other investigators. The automated suction and pressure unit, which manoeuvres the capsule, has done trouble-free service during 2 1/2 years.

S. Nordio (Genoa), A. Donath (Berne), F. Macagno (Berne) & R. Gatti (Genoa): *Some observations on Ca, Mg and Sr absorption in intestine*

The authors studied two problems: that of the relationships between Ca, Sr absorption, vitamin D and Na and that of the relationships between the absorption of Ca, Mg and Sr in intestine.

With regard to the first problem following observations were made:

1. In idiopathic hypercalcuria a prolonged treatment with hydrochlorothiazide chronically reduced urinary Ca-elimination, but in spite of this reduction it did not modify neither osteoporosis nor growth retardation.

2. The hypothesis that hydrochlorothiazide reduces Ca absorption in intestine was put forward and this hypothesis was confirmed by following observations:

(*a*) In normal children on constant diet hydrochlorothiazide reduces the urinary Ca-elimination and increases the faecal Ca-elimination; (*b*) hydrochlorothiazide does not increase the intestinal secretion of the intravenously injected $^{47}$Ca; (*c*) on the contrary, in normal children treated with this drug, a statistically significant reduction of $^{85}$Sr intestinal absorption was demonstrated.

3. Considering Binder's demonstration of an inhibiting effect of thiazides on Na transport in gut sac, other clinical investigations demonstrated:

(*a*) A certain reduction of $^{85}$Sr intestinal absorption in two normal children on poor Na diet; (*b*) an increase of Ca-elimination with stools in four normal children on either rich or poor Na diet; (*c*) a reduction of the effect of vitamin D on $^{85}$Sr absorption on hydrochlorothiazide treatment (this reduction was clear in 3 of 6 cases); (*d*) a reduction of the effect of vitamin D on $^{85}$Sr absorption on poor Na diet (this reduction was clear in 2 and less evident in 4 other cases).

With regard to the second problem the authors presented the results of the investigations performed in a case of chronic idiopathic hypomagnesemia with secondary hypocalcemia, in a control subject on prolonged poor Mg diet, in control subjects fed with a diet containing high doses of Mg and in a few cases of common and vitamin D resistant rickets. Following observations must be chiefly emphasized:

1. Chronic idiopathic hypomagnesemia with secondary hypocalcemia chiefly depends on a intestinal Mg malabsorption.
2. In the same disease $^{85}$Sr was also malabsorbed, while intestinal absorption of

stable Ca was only a little lower than in control subjects studied under the same conditions

3. A prolonged poor Mg diet reduces ⁸Sr intestinal absorption (Mg absorption was high)
4. In normal children Mg glycerophosphate oral load increases ⁸Sr and in a lesser degree ⁴⁷Ca intestinal absorption
5. In Ca malabsorption of rickets and in Mg-malabsorption the mitochondria of epithelial cells of intestinal mucosa obtained by peroral biopsy are swollen

P A Krasilnikoff & P Rødbro (Copenhagen) *Intrinsic factor secretion in early child hood*

Megaloblastic anemia in childhood can be due to different causes, and among these the deficiency of vitamin $B_{12}$ is one of the most interesting This deficiency is a consequence of a defective intestinal absorption of the vitamin in question and is due either to various intestinal disorders or to an abolished gastric secretion of intrinsic factor (IF)

These intestinal disorders all show a normal morphology of the gastric mucosa as well as a normal production of acid and IF The abolished gastric secretion of IF in childhood is most frequently accompanied by a normal gastric biopsy and secretion of acid (juvenile pernicious anemia) The adult type of pernicious anemia is very uncommon in children and is characterised by an atrophy of the gastric mucosa and a correspondingly abolished acid and IF secretion

In order to distinguish between these different types of vitamin $B_{12}$ deficiency in childhood it is therefore necessary to determine the IF secretion This can be done by a vitamin $B_{12}$ absorption test, by a bioassay but the most convenient method seems to be a direct estimation of IF in gastric juice We have by means of a radioimmunoassay determined the gastric secretion of IF in 8 normal children 9–30 months of age approximately the age of the debut for pernicious anemia in the juvenile form The children secreted from 1000–4000 units/hour of IF The secretion was significantly correlated to age body weight and body surface area Adults normally produce from 5000–25 000 units/hour, and adult patients with pernicious anemia less than 70 units/hour It thus seems that normal children secrete quite sufficient amounts of IF so that the measurement of gastric secretion of acid and IF can be used in the differential diagnosis of children with megaloblastic anemia caused by vitamin $B_{12}$ deficiency

C Polonovski & J Navarro (Paris) *Provoked hyperfolicemia as a test of intestinal absorption in children*

Provoked hyperfolicemia has been used in children as a test of intestinal absorption Two sorts of tests were performed, one without previous saturation and other after per os saturation Usual doses were 5 mg of folic acid per 1 73 m² of body surface area Blood samples were taken immediately before the test, at 2 and at 4 hours When previous saturation was indicated folic acid was given for five days (5 mg per day from 0 to 18 months, and ... mg after this age) and the test was performed 2 days after saturation

Maximum concentration was obtained at two hours in the simple test and at four hours in the saturated test Mean peak values were 20 mμg per ml from 0 to 1 year and 25 mμg per ml after one year With previous saturation values were higher 25 mμg per ml from 0 to 4 months 35 mμg from 4 to ... months and 40–50 mμg after 12 months In adults mean maximum values were 50 mμg in the simple test and 80 mμg in the test with saturation This test has been performed in ... cases of coeliac disease the folic acid level in serum were notably decreased mean value ... mμg per ml Low values were obtained in premature infants and in normal infants below

ge of 4 months too Comparison with the d xylose test showed perfect agreement

Provoked hyperfolicemia appears to be an interesting test in digestive syndromes and especially in coeliac disease in which the result apparently is significant after age of 4 months

June K Lloyd (London) *Faecal and serum lipids after medium-chain triglyceride feeding*

The effects of medium-chain triglyceride (MCT) diets have been studied in 13 children with malabsorption due to four groups of disorders obstructive jaundice (3) pancreatic insufficiency (3) a betalipoproteinaemia (2) and intestinal lymphangectasia (5) Steatorrhea was improved in all patients but analysis of faecal fatty acids showed that whereas the absorption of MCT was virtually complete in intestinal lymphangectasia and a betalipoproteinaemia (less than 1 of the ingested C 8 0 and C 10 0 fatty acids appearing in the faeces) absorption was less efficient in the absence of bile or pancreatic lipase (up to 4 of C 8 0 and up to 23 of C 10 0 fatty acids appearing in the faeces)

Serial estimations of serum lipids in 5 children during a 3 week period after the isocaloric exchange of MCT for dietary LCT (long-chain triglyceride) showed a small rise in serum triglyceride concentration and a prompt and sustained decrease in the proportion of linoleic acid and increase in palmitoleic acid in this fraction Similar changes were observed in 10 children receiving MCT diets for periods up to 28 months No significant changes occurred in total cholesterol or phospholipid concentrations but linoleic acid was decreased in phosphatidyl choline and nervonic acid was increased in sphingomyelin Only trace quantities of MCT fatty acids were found in any serum lipid fraction The fatty acid changes during MCT feeding are probably indicative of increased lipogenesis

J Rey & C Ricour (Paris) *Study of micellar and oil phases during hydrolysis of fat*

A preliminary study of hydrolysis of fat and micellar solubilisation has been done on three normal children After ingestion of a test meal (casein amylopectin sucrose and fat) the intestinal liquid was taken by syphon using a tube with a simple lumen placed in J 1 The fats used for this study were chosen so that equal proportions of the 6 fatty acids varied in proportion to the length of their carbon chains (from C 12 to C 18) and in the number of their double bonds (from 0 to 2) for fatty acids with 18 carbons Lipase was inactivated by heat (70 C for 10 minutes) Oil and micellar phases were separated after centrifugation at 75 000 g for 8 h at 37 C Lipids were extracted by a mixture of ether heptan alcohol and water Glycerides were separated by chromatography on a silicic acid column

In comparison with Hoffman & Borgstrom working on adults we have found a very different composition in the two phases The only components in the micellar phases were MG and FFA The oil phases also contained MG and FFA but the whole of TG and DG The respective importance of the two phases changed from one sample to another depending on the degree of the hydrolysis The percentage of MG is higher than FFA in micelles when compared to the total FFA and MG Therefore there were twice as many FFA as MG in micelles except at the end of the hydrolysis at this stage the ratio FFA/MG increased a great deal Finally we have consistently found a molar excess of FFA which probably indicates a faster absorption of the MG than of the FFA

H Kayden (New York) *Metabolism and absorption of fatty acids in puromycin and orotic acid fed rats*

The metabolism of short and long-chain fatty acids and triglycerides was compared in control and puromycin treated rats The drug had

stable Ca was only a little lower than in control subjects studied under the same conditions

3 A prolonged poor Mg diet reduces [illegible]Sr intestinal absorption (Mg absorption was high)

4 In normal children Mg glycerophosphate oral load increases [illegible]Sr and in a lesser degree $^{47}$Ca intestinal absorption

5 In Ca malabsorption of rickets and in Mg malabsorption the mitochondria of epithelial cells of intestinal mucosa, obtained by peroral biopsy are swollen

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A Prader D H Shmerling M Zachmann & Z Biro (Zurich) *Catch up growth in celiac disease*

Twenty nine patients with celiac disease diagnosed at the age of 0 6 to 2 years (mean 1 15 years) on the basis of steatorrhea xylose malabsorption and intestinal mucosal atrophy have been treated with strictly gluten free diet for 1 2 to 3 9 years (mean 2 53 years) Before treatment mean weight age was 43 mean height age 76 % and mean bone age about 73 % of chronological age Weight age reached full catch up i e 100 of chronological age in the second half of the first year height age in the second year and bone age at the end of the second or in the third year of treatment Preliminary results suggest that cortical thickness of the metacarpal bones is markedly reduced in the beginning catches up after 6 to 12 months and overshoots the mean normal values after 2 years

D H Shmerling (Zurich) *An analysis of controlled relapses in gluten induced coeliac disease*

The clinical course the biochemical changes and the morphological alterations of the intestinal mucosa were studied in 10 children with coeliac disease in remission under gluten free diet and during the re introduction of gluten Six patients went into full relapse presenting the typical clinical symptoms flat mucosa and a variable degree of absorption defects (xylose fat, N) The other 4 remained without any clinical or biochemical evidence of a relapse inspite of a successive deterioration of their intestinal mucosa progressing to complete flattening

In both groups of patients the ages at the institution of gluten free diet (mean 1 ¹¹/₁₂ and ¹¹/₁₂ ) and at the re introduction of gluten (mean 3 ¹⁰/₁₂ and 3 ⁴/₁₂ ) were similar and thus also the duration of dietary treatment (mean 2 ... and 2 ¹⁰/₁₂) The only differences between the groups was that full relapses occurred within 6 to 9 months whereas the other patients developed a flat mucosa only later and remained free of symptoms after 2–3 years off diet The conclusions drawn from this preliminary small series are

1 The lack of clinical symptoms of relapse does not exclude a response to gluten in these patients

2 All the patients studied responded to the re introduction of gluten by developing a flat mucosa

3 The course of events is remarkably slow and the very first signs of a response can appear 6 to 12 months after the re introduction of gluten

R Gruttner (Hamburg) *The effect of fractions from hydrolysate of gliadine in patients with coeliac disease*

By means of peptic and tryptic digestion of gliadine and ultrafiltration we obtained a mixture of peptides which was orally given to patients with coeliac disease This mixture showed a distinct effect with respect to manifestation of symptoms on patients with coeliac disease as earlier reported by Krainick and coworkers (1959) This positive effect on patients with coeliac disease could be proved after feeding the test material by the disturbed fatty acid absorption In continuation of these experiments we obtained a fraction of acid peptides by means of column chromatography of hydrolysate This fraction amounted to 7 of the total hydrolysate This mixture of peptides also caused the coeliac symptoms We succeeded in finding some single peptides in this fraction by thin layer chromatography

On the basis of these results the hypothesis of an antigen antibody reaction becomes less probable as the symptoms of coeliac disease are not only effected by the protein gliadine but also by peptides in a solution free from proteins Therefore it is more probable that a

no effect on the absorption of octanoic acid and of trioctanoin, presumably because short chain fatty acids are absorbed directly into the portal stream as albumin bound free fatty acid However long-chain fatty acid absorption into the thoracic duct lymph in the form of chylomicron triglycerides was inhibited by puromycin Nonetheless oxidation of the administered long chain fatty acid contained in the test meal to carbon dioxide was equal in puromycin treated rats and in control animals In thoracic duct cannulated animals treated with puromycin in the amount of expired label carbon dioxide derived from a test meal of long-chain lipids varied inversely to the amount of label in the lymph lipid Long chain fatty acids therefore can be absorbed directly into the portal venous system

Rats fed a semi synthetic diet containing orotic acid developed a fatty liver and low to absent levels of plasma betalipoprotein analysis of thoracic duct lymph suggested that the absorption of long-chain fatty acids and chylomicron formation were unimpaired The oxidation of orally administered labeled 1-14 C oleic acid to carbon dioxide was the same in control and in orotic acid fed animals There was a lesser degree of oxidation of orally administered labeled octanoic acid in orotic acid-fed animals than in control animals

W Plenert & U Spahn (Jena) *Effect of starvation and prolonged fasting on lipid metabolism in obese children*

Many difficulties are met in the treatment of obesity in childhood In the course of the last few years the use of prolonged fasting has been advocated and the effects on metabolism have been extensively studied in obese adults Corresponding studies on the metabolic changes in obese children undergoing absolute starvation are greatly lacking Lately the number of children with considerable overweight has increased remarkably For this reason we started our investigations on the results of starvation and prolonged fasting in obese children Our data comprise 70 children undergoing starvation (= no energy supply at all, water ad libitum and vitamins) or prolonged fasting (= mostly 400 kcal partly 600 kcal daily for school children) Some important observations can be summed up as follows

1 Starvation over a period of 10-14 days is an effective method for short term weight reduction in obese children, and may be repeated intermittently after periods of caloric restriction (i e 400 kcal/day) Such a regimen may be used without any danger, slight side reactions can be expected in about 4 % of the overweight children

2 The magnitude of weight loss depends on several factors Most important in this connection is the energy supply immediately before caloric restriction A hospital diet of 400 kcal/d resulted in a mean initial weight loss of about 0 5 kg/d During 10 days of absolute starvation the mean weight loss was 0 6 kg/d in the first 3 days and of 0 45 kg/d in the last 3 days of the period There was no correlation between weight loss and the degree of overweight

3 Before treatment the fasting level of serum free fatty acids in obese children was invariably raised (1 520 ± 0 515 mval/l) During caloric restriction (400 – 600 kcal/d) no significant rise of FFA could be observed but during absolute caloric deprivation the fasting levels of FFA rose to 2 10 ± 0 62 mval/l

4 Other fractions of serum lipids (esterified fatty acids cholesterol phospholipids and triglycerides) were in the normal range before caloric restriction In the period of absolute starvation there was a statistically significant fall of these serum lipid levels The percentage reduction was for esterified fatty acids 31 %, phospholipids 17 %, triglycerides 36 % and total cholesterol 24 % respectively The lower total cholesterol depended on esterified cholesterol of which the decrease in linoleic acid esters (45 %) represented the main part After refeeding with 400–600 kcal/d the serum lipid levels remained as low as in the period of starvation

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D. H. Shmerling (Zurich): *An analysis of controlled relapses in gluten induced coeliac disease*

The clinical course, the biochemical changes and the morphological alterations of the intestinal mucosa were studied in 10 children with coeliac disease in remission under gluten free diet and during the re-introduction of gluten. Six patients went into full relapse presenting the typical clinical symptoms, flat mucosa and a variable degree of absorption defects (xylose, fat, N). The other 4 remained without any clinical or biochemical evidence of a relapse inspite of a successive deterioration of their intestinal mucosa progressing to complete flat lesion.

In both groups of patients the ages at the institution of gluten free diet (mean 1 $^{?}/_{1}$ and $^{?}/_{1}$) and at the re-introduction of gluten (mean 3 $^{10}/_{1}$ and 3 $^{?}/_{1}$) were similar and thus also the duration of dietary treatment (mean 2 $^{?}/_{12}$ and 2 $^{10}/_{12}$). The only differences between the groups was that full relapses occurred within 6 to 9 months whereas the other patients developed a flat mucosa only later and remained free of symptoms after 2–3 years off diet. The conclusions drawn from this preliminary small series are:

1. The lack of clinical symptoms of relapse does not exclude a response to gluten in these patients.
2. All the patients studied responded to the re-introduction of gluten by developing a flat mucosa.
3. The course of events is remarkably slow and the very first signs of a response can appear 6 to 12 months after the re-introduction of gluten.

R. Gruttner (Hamburg): *The effect of fractions from hydrolysate of gliadine in patients with coeliac disease*

By means of peptic and tryptic digestion of gliadine and ultrafiltration we obtained a mixture of peptides which was orally given to patients with coeliac disease. This mixture showed a distinct effect with respect to manifestation of symptoms on patients with coeliac disease as earlier reported by Krainick and coworkers (1959). This positive effect on patients with coeliac disease could be proved after feeding the test material by the disturbed fatty acid absorption. In continuation of these experiments we obtained a fraction of acid peptides by means of column chromatography of hydrolysate. This fraction amounted to 7 % of the total hydrolysate. This mixture of peptides also caused the coeliac symptoms. We succeeded in finding some single peptides in this fraction by thin layer chromatography.

On the basis of these results the hypothesis of an antigen antibody reaction becomes less probable as the symptoms of coeliac disease are not only effected by the protein gliadine but also by peptides in a solution free from proteins. Therefore it is more probable that a

defect of an enzyme might be the cause of the defective cleavage in the metabolism of gliadine in the cells of the intestinal mucosa and its destruction

J Jos (Paris) *Immediate effects of gliadin on the intestinal mucosa in coeliac disease*

In five young patients with coeliac disease, treated for many years we have been able to produce a rapid histologic reaction of gluten The following procedure was used after two or three years of treatment biopsies were obtained showing normal appearance wheat was administered orally and some hours or days later repeated biopsies were taken from the same position in the distal duodenum

Broad villi increase of cell number in the lamina propria are the first changes followed by flattening of epithelial cells and, sometimes a complete loss of the epithelium In one child pathological changes are apparent within 6 hours in another the lesions are very slight after one week of normal gluten containing diet and become obvious only one month after the onset of this diet

No clinical intolerance or steatorrhea was observed, except in one patient who developed diarrhea on the twelfth day of the diet

Such experiments can be carried out only when the diagnosis of coeliac disease is firmly established and the mucosa has returned to normal

R Gruttner (Hamburg) *The nonspecific effect of a glutenfree diet in steatorrhea of children*

In some cases of malabsorption syndrome with moderate steatorrhea we observed after the administration of a glutenfree diet an amelioration of the symptoms even in those cases which were not caused by an intolerance of gluten As in some cases even the excretion of fatty acids decreased significantly we may consider this to be an unspecific effect of the glutenfree diet on the steatorrhea not induced by gluten Presumably only in few cases these experiences will have therapeutic consequences But on the other side these observations show that coeliac disease cannot be diagnosed on the basis of the favourable effect of a glutenfree diet As we have not yet sufficient knowledge about the pathophysiology of gliadine as a pathogenetic factor all explanations of the nonspecific effect of a gliadine free diet in other forms of intestinal insufficiency can only be of hypothetic character We know that gliadine in comparison with other proteins needs a longer time for hydrolysation which could be explained by its special structure Therefore it is possible that there is a connection between the prolonged hydrolysis of the gliadine and its pathogenicity in coeliac disease on one hand and its nonspecific effect on the other hand

J K Visakorpi (Helsinki) *Cow s milk as a cause of malabsorption syndrome*

In a study concerning children suffering from prolonged diarrhoea, clinical intolerance to cow s milk was found in 33 patients during a period of six years The mean age of these patients was 2–3 months at the onset of symptoms and prolonged diarrhoea vomiting and failure to thrive were the main symptoms Classic allergic symptoms, such as atopic eczema were observed in every fifth patient Special investigations revealed more or less severe absorption defects duodeno–jejunal mucosal villous atrophy circulating precipitins to cow s milk and elevated serum IgA content The feeding of cow s milk caused either a rapid reaction with vomiting diarrhoea and even collapse in few hours or a slower reaction simulating the symptoms typical for coeliac disease The clinical sensitivity to cow s milk was transient ameliorating usually at the age of 10–12 months When discussing the pathogenesis of cow s milk intolerance malabsorption of disaccharides as the primary cause was conclusively excluded The harmful fa[illegible] of cow s milk in

hes cases apparently belonged to the protein fraction Some evidence was obtained that foreign protein may directly damage the intestinal mucosa apparently by an immunological mechanism However the basic aetiology of this disease remains open

H Loeb G van de Velde & E Brachet (Brussels) *Transient enteropathy and hypoproteinemia in an infant*

The case report concerns a male infant who developed a syndrome characterized by bloody diarrhoea weight loss and edema hypoproteinemia anemia and eosinophilia The clinical manifestations appeared as early as the second day of life when breast fed and they persisted after the introduction of a cow s milk formula on the seventh day

At one month of age X rays of the small bowel demonstrated signs of a non specific jejunitis A nitrogen balance disclosed the existence of an important intestinal protein loss A jejunal biopsy showed subtotal villous atrophy with chorionic infiltration by numerous lymphocytes and plasma cells The in vitro study of a fragment of jejunal mucosa disclosed a decrease of the active transport of glucose and leucine

A lipid free diet was followed by the normalization of the stools and a spectacular improvement of the clinical and laboratory findings The introduction at 6 weeks of age of medium-chain triglycerides did not alter the favourable course At 2 1/ months of age the albumin metabolism was studied with intravenously injected RIHSA The substitution on the 12th day of the test of a common cow s milk formula did not change the turnover rate of injected albumin At 3 1/ months of age radiology of the small bowel and a nitrogen balance proved normal histological control still revealed subtotal villous atrophy whereas cell infiltration was decreased

The existence of an exsudative enteropathy was considered Although the rapid clinical improvement prohibited the collection of evidence for such a process this observation is comparable with those which enter into the syndrome of protein losing enteropathy

Clinical picture as well as laboratory radiological and histological findings led us to consider that our infant had presented a process of transient allergy of unknown nature at the level of jejunal mucosa

*G W Meeuwisse*

# THE FINNISH PEDIATRIC SOCIETY

## Meeting September 11 1968

H Bickel (Heidelberg) *Inborn errors of metabolism associated with brain damage — Recent advances in early detection and treatment*

Research into the causes of brain damage is of decisive importance for their diagnosis and prevention Present day information suggests that a large although still unknown percentage of brain damage in early childhood is due to enzymopathies Hereditary enzyme defects usually inherited as an autosomal recessive trait, lead to metabolic errors disturbing cerebral function and development The error may involve very different pathways of the amino-acid carbohydrate fat, electrolyte water plasma protein hormone and bilirubin metab-

defect of an enzyme might be the cause of the defective cleavage in the metabolism of gliadine in the cells of the intestinal mucosa and its destruction

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Such experiments can be carried out only when the diagnosis of coeliac disease is firmly established and the mucosa has returned to normal

R Gruttner (Hamburg) *The nonspecific effect of a glutenfree diet in steatorrhea of children*

In some cases of malabsorption syndrome with moderate steatorrhea we observed after the administration of a glutenfree diet an amelioration of the symptoms even in those cases which were not caused by an intolerance of gluten As in some cases even the excretion of fatty acids decreased significantly we may consider this to be an unspecific effect of the glutenfree diet on the steatorrhea not induced by gluten Presumably only in few cases these experiences will have therapeutic consequences But on the other side these observations show, that coeliac disease cannot be diagnosed on the basis of the favourable effect of a glutenfree diet As we have not yet sufficient knowledge about the pathophysiology of gliadine as a pathogenetic factor all explanations of the nonspecific effect of a gliadine free diet in other forms of intestinal insufficiency can only be of hypothetic character We know that gliadine in comparison with other proteins needs a longer time for hydrolysation which could be explained by its special structure Therefore it is possible that there is a connection between the prolonged hydrolysis of the gliadine and its pathogenicity in coeliac disease on one hand and its nonspecific effect on the other hand

J K Visakorpi (Helsinki) *Cow s milk as a cause of malabsorption syndrome*

In a study concerning children suffering from prolonged diarrhoea, clinical intolerance to cow s milk was found in 33 patients during a period of six years The mean age of these patients was 2–3 months at the onset of symptoms, and prolonged diarrhoea vomiting and failure to thrive were the main symptoms Classic allergic symptoms such as atopic eczema were observed in every fifth patient Special investigations revealed more or less severe absorption defects duodeno–jejunal mucosal villous atrophy circulating precipitins to cow s milk and elevated serum IgA content The feeding of cow s milk caused either a rapid reaction with vomiting diarrhoea and even collapse in few hours or a slower reaction simulating the symptoms typical for coeliac disease The clinical sensitivity to cow s milk was transient ameliorating usually at the age of 10–12 months When discussing the pathogenesis of cow s milk intolerance malabsorption of disaccharides as the primary cause was conclusively excluded The harmful factor of cow s milk in

[...]ese cases apparently belonged to the protein [...]action Some evidence was obtained that [...]ocrein protein may directly damage the intes[...]nal mucosa apparently by an immunological [...]echanism However the basic aetiology of [...]his disease remains open

H Loeb G van de Velde & E Brachet (Brussels) *Transient enteropathy and hypoproteinemia in an infant*

The case report concerns a male infant who developed a syndrome characterized by bloody diarrhoea weight loss and edema hypoproteinemia anemia and eosinophilia The clinical manifestations appeared as early as the second day of life when breast fed and they persisted after the introduction of a cow s milk formula on the seventh day

At one month of age X rays of the small bowel demonstrated signs of a non specific jejunitis A nitrogen balance disclosed the existence of an important intestinal protein loss A jejunal biopsy showed subtotal villous atrophy with chronic infiltration by numerous lymphocytes and plasma cells The in vitro study of a fragment of jejunal mucosa disclosed a decrease of the active transport of glucose and leucine

A lipid free diet was followed by the normalization of the stools and a spectacular improvement of the clinical and laboratory findings The introduction at 6 weeks of age of medium-chain triglycerides did not alter the favourable course At 2 1/ months of age the albumin metabolism was studied with intravenously injected RIHSA The substitution on the 12th day of the test of a common cow s milk formula did not change the turnover rate of injected albumin At 3 1/ months of age radiology of the small bowel and a nitrogen balance proved normal histological control still revealed subtotal villous atrophy whereas cell infiltration was decreased

The existence of an exudative enteropathy was considered Although the rapid clinical improvement prohibited the collection of evidence for such a process this observation is comparable with those which enter into the syndrome of protein losing enteropathy

Clinical picture as well as laboratory radiological and histological findings led us to consider that our infant had presented a process of transient allergy of unknown nature at the level of jejunal mucosa

*G W Meeuwisse*

# THE FINNISH PEDIATRIC SOCIETY

Meeting September 11 1968

H Bickel (Heidelberg) *Inborn errors of metabolism associated with brain damage—Recent advances in early detection and treatment*

Research into the causes of brain damage is of decisive importance for their diagnosis and prevention. Present day information suggests that a large although still unknown percentage of brain damage in early childhood is due to enzymopathies Hereditary enzyme defects usually inherited as an autosomal recessive trait, lead to metabolic errors disturbing cerebral function and development The error may involve very different pathways of the amino-acid carbohydrate fat electrolyte water plasma protein hormone and bilirubin metab-

olism More than 40 examples of such disorders are already known Early detection and biochemical characterisation in 17 types have enabled the clinician to develop a more or less effective treatment and at a sufficiently early stage to prevent their manifestations The rapidly growing brain of the young child seems especially liable to be affected by such metabolic deviations so that early diagnosis is of decisive importance in preventing brain damage before it becomes irreversible

A number of screening tests are now available to detect metabolic oligophrenia They should be sufficiently specific sensitive and simple for the purpose These criteria are well met by the microbiological inhibition tests of Guthrie which demonstrate in a drop of blood taken from a heel prick of every newborn infant the phenylalanine increase of phenylketonuria, the leucine increase of maple syrup disease, the methionine increase of homocystinuria the galactose increase of galactosaemia etc Of the other screening tests one and two dimensional thin layer chromatography with blood and urine are especially suitable to detect the increased concentration of the various aminoacids and sugars in some of these diseases Our technique has been described in a recent communication (Bremer, Nutzenadel & Bickel Dunnschichtchromatographische Methoden zur Erkennung von Aminoacidurien Aminoacidamien sowie der Galaktosamie *Mschr Kinderheilk* in press)

A procedure is now being developed for the elution of the aminoacids present in the blood drops of the Guthrie filter paper card for one dimensional thin layer chromatography which will greatly facilitate the specimen collection in every newborn child

The more effective the screening method and detection of these conditions the more important it becomes to develop a successful treatment Specific diets have been elaborated to correct the metabolic deviation and to prevent brain damage in phenylketonuria, maple syrup disease, homocystinuria, hyperammonaemia hyperglycinaemia, argininosuccinicaciduria ornithinaemia (a newly discovered inborn metabolic error) and some other diseases Rapid further progress is to be expected in this field The expenditure of time and money involved in the detection and prevention of inborn metabolic errors is far less than that involved in the case of the late or undiagnosed patient, apart from the emotional stress which they inflict upon their families

For a detailed account of this topic and the literature see

Bickel H & Cleve H Handbuch der Humangenetik vol V/2 (ed Becker P E) Thieme Stuttgart 1967

Bickel H Some recent advances in inborn errors of metabolism (ed Holt K S and Coffey V P) p 39 Livingstone Edinburgh

## NEW BOOKS RECEIVED

... Hart ( *Physiologie und Pathologie der Pu* ... Pädiatrische Fortbildungskurse für die Praxis ... 23 123 pp illus S Karger Basel & New York ... sFr/DM 39

Hungerland (ed ) *Die Betreuung des diabetischen* ... 607 pp illus Ferdinand Enke Verlag Stutt ... 1968 DM 25

O'Brien, F A Ibbott & D O Rodgerson *Laboratory manual of Pediatric Microbiochemical Techniques* 4th Ed 384 pp Hoeber Medical Division, Harper & Row New York 1968 $11 90

I Gontrea & P Sutescu *Natural antinutritive substances in foodstuffs and forages* 184 pp S Karger Basel & New York 1968 sFr/DM 43 40

L Meyler & H M Peck (eds ) *Drug induced diseases* Vol 3 340 pp Excerpta Medica Foundation Amsterdam 1968 $20 00

G Fanconi *The history of the International Pediatric Association* 129 pp illus Schwabe & Co Publ., Basel & Stuttgart 1968 Fr 17 50

## BOOK REVIEWS

Yvonne Brackbill (ed ) *Infancy and early childhood A handbook and guide to human development* 523 pp Collier Macmillan London 1967 84s

This book is of interest foremost to psychologists in research work on infant development in all its aspects They have here a handbook and useful bibliography with up to date reviews of most areas of infant development Conditioning and learning is reviewed by Brackbill and the Russian psychologist Koltsova with incorporation of much interesting research by Russian workers This is perhaps the most original but also least comprehensive review excluding e g the social learning oriented research and frame of reference of R Sears, Bandura and Walters Language development, cognition and development of social behavior are well presented but one often feels dissatisfied by sketchy reviews of a large body of empirical research and a lack of more comprehensive theory-guided frames that indeed warranted the research There is a fear in too much theory and speculation well-known in child psychology but it is danger in too loose empirical research too The chapter on emotional behavior and personality development shows the difficulties in synthesis It is symptomatic that three authors write in rather descriptive approach psychoanalytic theories and the biological approach but it is also true that prospects of integration are shown e g by behavior genetics that indeed should be worth a more penetrating review than that in this book Of most obvious interest for pediatricians are the chapters on infant motor development by D H Crowell on sensory and perceptual processes by W C Spears and R H Hohle and especially the penetrating and well organized chapter on developmental psychophysiology by A Steinschneider with illuminative looks into the genesis of psychosomatic deviations The age range reviewed in infant development research mostly up to 4 years and the emphasis is on normal development deviations and pathology is seldom elaborated The book has a supplement by "Behavior in infancy and early childhood A book of readings" by 63 reprinted original articles for psychology students and adapted as a course of advanced teaching in infant development One feels that a chapter on test methods on infants and on research methodology would have made the handbook still better

*Anders Torold*

E Rossi & E Stoll (eds ) *Cystic Fibrosis* Proceedings of the 4th International Conference on Cystic Fibrosis of the Pancreas (Mucoviscidosis) 1966 Part I 404 pp 182 figs 80 tab S Karger AG Basel/New York 1967 sFr/DM 85 —

In September 1966 the 4th International Conference on cystic fibrosis of the pancreas was held in Bern/Grindelwald The first part of the proceedings is published in this book (The second part concerning the biochemistry of the glycoproteins is published separately ) This book contains 46 papers on various problems of this puzzling disease Each paper is fol

olism More than 40 examples of such dis orders are already known Early detection and biochemical characterisation in 17 types have enabled the clinician to develop a more or less effective treatment and at a sufficiently early stage to prevent their manifestations The rapidly growing brain of the young child seems especially liable to be affected by such metabolic deviations so that early diagnosis is of decisive importance in preventing brain dam age before it becomes irreversible

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Bickel H Some recent advances in inborn errors of metabolism (ed Holt K S and Coffey V P) p 39 Livingstone Edinburgh

illustrated with figures Then the biochemical disturbances of the metabolism of each amino acid are explained in detail The methods used for detection of these abnormalities are briefly described For each amino acid a reference list is given which makes the chapter easy to survey

The third chapter entitled "Syndromes pathologiques aux perturbation metaboliques des divers amino-des deals with different clinical syndromes connected to disturbances of one amino acid or of one group of metabolically related amino acids which are treated as one unit Each disturbance has its own reference list This has the advantage of making the list short but some double references with the preceding chapter cannot be avoided These references are not listed alphabetically which would have been desirable

The following chapter is an attempt to give signs and symptoms of various diseases known to be related to impairments in the amino acid metabolism Typical features are for example the smell of the urine and speech troubles The reviewer does not understand the value of this chapter So many different clinical symptoms can occur in a patient with a metabolic disorder that it would be better to look for the amino acids with a simple screening procedure than to look up all the possible syndromes in this chapter This chapter ends in a table which covers 27 different "amino acidopathies and 24 different symptoms Seven of 27 pathological conditions are not related to mental impairment This means that the title of the book is somewhat misleading

In general the authors do not take any position or judgement on the very often controversial and therefore confusing biochemical finding reported as related to an impairment in amino acid metabolism But this should be interpreted as positive because of the difficulties often involved in judging the material of other investigators especially concerning mental disturbances

The fifth chapter is called Diagnostique biologique and gives some facts of an area which could be regarded clinical chemistry One section deals in greater detail with problems encountered with hyperphenylalaninemia and *related subjects such as a typical* phenylketonuria Since the detection of phenylketonuria is one of the most important task of a pediatrician dealing with metabolic disorders of amino acids it is justified to treat this subject in detail Problems dealing with the dietary treatment of phenylketonuries are also given in section followed by tables with the composition of food especially with regard to the phenylalanine content It would have been desirable to let such book end with a table containing the normal values for amino acids in urine and plasma from adults and children of different ages

The book reads relatively easily and seems to be a good source of references The references are followed up to 1967 The book certainly deserves a place on the desk of French speaking pediatrician or physician interested in the field of amino acidopathies

*W von Studnitz*

E. Rossi (ed): *Orthopaedische Fragen in der Pädiatrie Pädiatrische Fortbildungskurse für die Praxis* vols. 5–6 2nd ed 198 pp Karger Basel & New York 1968 sFr/DM 39

The paediatrician is often faced with various orthopaedic problems typical of the neonatal period of infancy of school age and of puberty The commonest and most important of these problems are dealt with in *Orthopaedische Fragen in der Pädiatrie* constituting volumes 5 and 6 of the series *Pädiatrische Fortbildungskurse für die Praxis* Nine surveys of the commonest deformities of the limbs and organs of locomotion in childhood are given by Swiss orthopaedists Half of the articles are written in German and half in French

The introductory article is concerned with congenital dislocation of the hip and is written by Professor M E Muller of Bern A good survey is given of the incidence aetiology diagnosis and principles of treatment in different age groups In the discussion of the various theories of the aetiology of the condition however no mention is made of the one we consider important the hormone theory The principles of treatment are mainly the same as those used in Sweden Brief descriptions are given of various surgical operations

Other classical orthopaedic diseases of childhood such as Perthes disease and other aseptic bone necroses (Prof L Nicod) and slipping of the upper femoral epiphysis (Prof W Tillard) are discussed in various articles Professor Tillard also contributes with an article on the normal and pathological development of the organs of locomotion The treatment of Perthes disease recommended by Professor Nicod is harder than what is the rule in Sweden He recommends 12–18 months bed rest with extension followed by the use of a none weight bearing brace for a roughly equal period The various causal factors of slipping of the epiphysis are discussed as well as the *significance of persistent dislocation* in the development of arthrosis is elucidated with figures from Jerre's thesis according to which all patients have more or less severe symptoms of arthrosis by the time they reach *45 years of age* The importance of early diagnosis and treatment is stressed

Scoliosis and Scheuermann's kyphosis are the subjects of separate articles The article on the latter disease includes interesting *information* on the pathoanatomy and diagnosis of the condition but a shorter and more concise presentation would have been more readable

Deformities of the foot belong to the commonest deformities of childhood These conditions are covered in an excellent way in a survey including a discussion of the diagnosis and treatment of various types of flat footedness

The volume also contains an article on growth disorders following traumatic injuries of the epiphyses *But these* problems fall within the field of limb surgery rather than in that of paediatrics

*The* book appears to be a good guide for the paediatrician in the evaluation of various deformities

lowed by references and by a discussion which not seldom is very informative

As a proof of the current interest in research on this disease no less than 12 papers are dealing with the physiology and pathophysiology of serous secretion of the sweat and salivary glands Dr Slegers gives a mathematical approach to the two step reabsorption hypothesis in the sweat gland duct and Dr Emrich *et al* present in a preliminary report results suggesting that the sodium reabsorption from an isotonic precursor fluid is defect in cystic fibrosis

In a round table conference on recent advances in cystic fibrosis Drs Shwachman and Mahmoodian give us valuable information on their great experience on pilocarpin iontophoresis sweat test which was carried out on 920 patients and on 4269 control individuals

One chapter contains 15 papers on various clinical investigations on cystic fibrosis e g pulmonary function iron absorption the use of micro-sodium electrode in the diagnosis electrolyte concentration in the nails In this chapter Dr Spock *et al* present their interesting *in vitro* study on ciliar motility It was found that a serum factor in patients with cystic fibrosis caused asynchronous beating of cilia of tracheal explants

In the therapy chapter Dr Matthews *et al* present their intensive pulmonary treatment infants are placed in mist tents on aerosol therapy and postural drainage as soon as the diagnosis is settled and in some cases as early as the first day of life It is of interest to note that in the discussions in this chapter several authorities point out that N acetyl cysteine is of questionable value and they do not recommend it

The different papers are more or less readable and the illustrations mostly of good quality For the pediatrician this book contains valuable information

*Tor Lindberg*

Jacques Gerbeaux *Tuberculose Primaire de l Enfant* 284 pp 51 figs Éditions Médicales Flammarion Paris 1967 70 F

This book gives a fairly good information of the French contributions to the study of tuberculosis in children Of special value and historical interest are the reports of Robert Debre Léon Bernard and other French authors regarding the early manifestations of tuberculous infection in infants isolated from their infectious mothers At that time there was an almost unanimous opinion of the absolute fatal outcome of infection in infants This concept has now changed to a more optimistic view thanks to French German and Scandinavian authors Gerbeaux who has studied early tuberculosis in childhood since many years has published this volume in due time primary tuberculous infection having become rather rare in unvaccinated children and after the establishment of the diagnosis chemotherapy changes the clinical picture and heals the disease It is therefore rath r seldom that we at least in Western and Northern F have the opportunity to study an uncomplicated and untreated case of primary tuberculosis For m th book in an historical document and as such exceller and up to date

*Arvid Wallgren*

S G Clayton (ed ) *Obstetrics Some current problems* 9? pp illus British Medical Bulletin *24* No 1 London 1968 40s

This bulletin summarizes some of todays knowledge in the field of maternal placental foetal relationship It contains 14 papers The emphasis is on recent research and has application to the management of cases One paper is concerned with different aspects on folate metabolism and reproduction Others deal with water and electrolytes cardiovascular dynamics blood coagulation disorders renal and liver diseases during pregnancy The positive advantages of screening pregnant women for bacteriuria are expressed in the paper concerning asymtomatic bacteriuria during pregnancy Of particular interest to pediatricians is the excellent review of prophylaxis of rhesus iso-immunization In the interesting survey on obstetric anaesthesia and analgesia it is said that paracervical block seems to be safe for mother and child However at the 1st Perinatal Congress in Berlin (196? Saling and Teramo independently reported on foetal acidosis and bradycardia during paracervical block The diagnostic advantages of ultrasonic echo sounding in obstetrics are described and the paper contains perfect illustrations As also pointed out in the rev[illegible] placental insufficiency ultrasonic investigation may give vital information concerning foetal growth

This volume is highly recommended to all pediatricians with interest in perinatal medicine

*Bengt Persson*

P Mozziconacci J Boisse A Lemonnier & C Charpentier *Les maladies métaboliques des acides aminés avec arriération mentale* 393 pp L Expansion Scientifique Paris 1968 51 Fr

The first part of this book dealing with impairment in amino acid metabolism is a very short introduction to the enormous field of amino acid chemistry It gives the chemical structure and chemical relationship of the important amino acids The fundamentals and principles of the metabolic breakdown of the amino acids are described This chapter is simple and informative since the authors limit the subject to major pathways of amino acid metabolism in man The next chapter is entitled Bases biochimiques et analytiques de l exploration de métabolisme des acides aminés It covers physiological and pathological metabolism of each amino acid and the pathways are divided in m[illegible] It is well

of the organs of locomotion in childhood The articles are well arranged and readable and are often accompanied by useful illustrations especially the article on dislocation of the hip and slipping of the femoral epiphysis

Since the chapters are independent articles written by different authors there is as in most books of this type a certain unavoidable degree of overlapping

*Åke Ahlberg*

Richard Torpin *Foetal Malformation Caused by Amnion Rupture during Gestation* 165 pp Charles C Thomas Publ Springfield Illinois 1968 US $11 50

A separate rupture of the fetal amnion membrane during gestation may leave the chorion intact and does not prevent the pregnancy to proceed until term The ruptured amnion can form fibrous strings which may create constriction or even amputation of fetal fingers toes or whole limbs If the fetus swallows the free part of such a string its face will be firmly attached to the placenta surface and the string may dig deeply into the fetal face thus causing a facial fissure originating from the mouth and not frequently following the natural embryological grooves Another consequence of a separate amnion rupture during gestation is the denudation of the chorionic membrane This hypothetically results in a loss of amniotic fluid through the sac wall leading to oligohydramnios The reduced volume of the sac inhibits the free development of the fetal limbs and may be a mechanical cause of deformities like clubfoot and club hand

The occurrence of this uncommon but interesting chain of events during fetal life is reviewed in a recent monography The author has collected 400 cases from the literature with fetal defects and co existing amnion rupture added 14 own observations and presented them in a richly illustrated volume In his enthusiasm for the subject the author sometimes seems to draw some bold conclusions regarding the connection between the reported findings but it does not diminish the value of this attractive book It is important that the etiology of these fetal injuries is recognized as the incidence of amnion rupture during gestation is estimated to be one in 5 000 to 15 000 human pregnancies It is not possible to establish any preventive measures but it is of vital concern that some bizarre fetal malformations can get a definite explanation so that exogenous teratogenic factors can be excluded

The subject of this book gives an impuls for the clinician to scrutinize the sac membranes in case of unusual defects in the newborn child

*Gerhard Gennser*

K Ebel & E Willich *Die Röntgenuntersuchung im Kindesalter Technik und Indikation* 739 pp Springer Verlag Berlin Heidelberg & New York 1968 DM 118

The book is concerned with the technique of roentgen examination in infancy and childhood with emphasis on the every-day examinations required in a non-specialized X ray department It also includes such rather sophisticated procedures as encephalography and ventriculography whereas angiocardiography for example is omitted on the ground that this examination is usually reserved for special centres of cardiology

An introductory part of the book deals with the general problems of pediatric radiology and recommends various methods for X ray protection and for immobilisation and fixation of the child in suitable positions This is followed by a systematic survey of examination procedures most of which are meticulously described and well illustrated The numerous reproductions of photographs and radiographs are of outstanding quality The description of each procedure is accompanied by a comment on its indications and sometimes contraindications and a concluding part of the book gives a list of the examination methods recommended by the authors in the investigation of certain diseases or symptoms

The procedures described are on the whole well established though many of them may and should be modified by the radiologist according to his personal experience and available equipment Similarly the contrast media recommended for various examinations constitute a good choice but others may be at least equally suitable These reservations serve more as a reminder that there may be several ways of arriving at a successful roentgen diagnosis and less as criticism of the book which certainly deserves to be included in the library of an X ray department

*G Theander*

G Joppich & F J Schulte *Neurologie des Neugeborenen* 589 pp Springer Verlag Berlin Heidelberg & New York 1968 DM 138

This thick book containing 452 pages text and 434 references covers with German thoroughness most of the neurological diseases which may give symptoms in the newborn infant Many other conditions are however also mentioned and discussed conditions which have no relation to the neonatal period This adds to the interest of the book but also to its volume and perhaps to the difficulties in finding exact and concise information about problems covered by the title of the book

The difficulties are enhanced by the organisation of the reference list which is given in alphabetical order at the end of the book The reader would have greatly appreciated a small list of the appropriate references after each chapter this would have made it possible for him to find new information about

Acta Paediat Scand 58 321–329 1969

# BALANCE OF NET ACID IN GROWING INFANTS

## Endogenous and Transintestinal Aspects

J KILDEBERG K ENGEL and R W WINTERS

From the Department of Pediatrics College of Physicians and Surgeons of Columbia University and Babies Hospital Columbia-Presbyterian Medical Center New York City N Y USA

In clinical practice diagnosis and treatment of metabolic acid base disturbances are usually based on continual observation of acid base variables of blood but systematic evaluation of deviations in such variables in terms of component contributions by primary and secondary changes in balance distribution and solvent concentration are rarely attempted It is the purpose of the present paper to introduce a chemically sound concept of *balance of net acid* in the pediatric literature and to present a series of studies of the net acid balance (NAB) in healthy premature infants The results serve to define the magnitude of the variables of the net acid input in growing infants as well as to establish explicitly the role of hydroxyapatite build up in determining the NAB during periods of active skeletal growth In addition they suggest a new view on the contribution of the gastrointestinal tract to physiological acid base homeostasis

### CONCEPTS AND DEFINITIONS

In the medical literature the term acid base balance has often been used indiscriminately In the present context the term "balance will be specifically defined as the difference between *input* and *output* and the term acid will be used to denote *net titratable acid* (base equivalents carrying a negative sign) Thus the *net acid balance* of the whole body or of some specified compartment of the body fluid over a given period of time represents net gains of titratable acid on titration to an arbitrary end point at pH 7 40 ($P_{CO}$ = zero mm Hg and temperature = 37 C) By this definition the separation of output from input becomes quite arbitrary and a choice may be made on purely physiological grounds Conventionally the output is defined as the *renal net acid excretion* Hence the input represents the net gain of titratable acid *from all extrarenal sources* Finally the term net acid requires further qualification From a physiological point of view three classes of acid (H donors) may be distinguished (1) carbonic acid which may be removed (in effect) from the body by pulmonary ventilation (2) metabolizable organic acids (e g lactic acid and ketoacids) and (3) all other acids Evidently the *net extrarenal* gain of acid belonging to the third category represents a (positive or negative) load of acid for which the urine is the only available route of excretion This quantity of acid is by definition the net acid input.

Two general types of contributions to the net acid input, so defined can be distinguished one type representing net gains by the body as

# ANNOUNCEMENTS

The Congress of French Speaking Pediatricians will be held at Strasbourg (France) on the 1st 2nd 3rd September 1969

The themes proposed are as follows Feeding of the Premature Infant Reanimation of the Newborn Baby Mucopolysaccharidosis and Sphingolipidosis For further information please apply to Professeur Agrege Daniel Willard Clinique de Pediatrie et Puericulture 67 Strasbourg (France)

Under the auspices of the International Pediatric Association the Greek Pediatric Society in collaboration with the Middle East and Mediterranean Pediatric Society is organizing in Athens from September 28th to October 1st 1969 the VI Mediterranean Middle Eastern Pediatric Congress For additional informations please write Greek Pediatric Society P O B 1519 Athens Greece

The Third International Congress on Neuro-Genetics and Neuro-Ophthalmology is being organized under the auspices of the World Federation of Neurology and particularly by its Research Committee It will be held in Brussels from the 25th to the 29th of August 1970

The themes of this Congress will be Amino acid pathology and Agammaglobulinemias The official languages will be English and French Any of you who would like to participate in this Congress or who would like to receive further information please address yourselves to Professor Pierre Danis 15 Avenue de la Folle Chanson Brussels 5 Belgium Prof Danis is secretary of the organizing committee

such, and the other representing net gains by the body fluid charging the kidney but not primarily affecting the total body content of acid or base To the first category belong contributions by oral intake (or vomiting) fecal excretion parenteral therapy endogenous production from neutral precursors (e g sulfuric acid originating by oxidation of sulfur-containing aminoacids) and titratable non oxidizable acid stranded in the body as a consequence of urinary loss of the conjugate anion of organic acid produced in the body To the second category belong titratable acid or base released in the body water as a result of ongoing skeletal mineralization or bone resolution respectively as well as any similar contributions to the net load on the kidney occurring by incorporation of phosphate residues into structural tissue proteins or lipids or by peptide bond formation and subsequent buffering of new protein It may be pointed out that such a distinction between retentional and distributional contributions and, hence between balances of net acid in the body ( body balance ) and balances of net acid in the body water ( body water balance ) is to some extent arbitrary but it serves the useful function of separating irreversible processes (first category) from reversible processes (second category)

If net acid balances for extracellular fluid or for plasma were to be calculated redistribution of H between intra and extracellular water and between interstitial fluid and plasma respectively would have to be taken into account Also it should be recognized that changes in the net acid concentration of the plasma bringing about a renal response may occur at a net zero input i e in the absence of changes in the net acid content of any body water compartment This occurs characteristically with dilution acidosis and contraction alkalosis (Winters et al (27) Cannot et al (3)) see also p 323 Similarly changes in plasma $P_{CO_2}$ and in the plasma concentration of oxidizable organic acids may influence the renal net acid excretion and hence indirectly the NAB

## PRINCIPLES OF INPUT ASSAY

The various physiological processes which contribute directly to the NAB of the growing infant are summarized in Table 1 where $H^+$ and $-H$ in the righthand column denote gains of titratable acid and base, respectively

Whereas measurement of the renal net acid excretion is relatively easy the quantitative characterization of the daily input poses formidable problems Within recent years, studies by Relman et al (22) and Lennon et al (14) have gone far toward resolving the problems of measurement of input in the non growing adult, and valuable balance data have been gained (5 12, 13) However experience with quantitative NAB measurements is still limited and to our knowledge the NAB of the growing organism has never been approached in these terms In order for NAB data on growing infants to be interpretable the available techniques must be modified to provide specified quantitative information concerning any contribution to the balance arising from the processes of growth Thus changes in the net load of acid presented to the kidney due to deposition of base in the growing skeleton as well as any similar effect attributable to growth of the soft tissues must be estimated

During the process of skeletal mineralization hydroxyapatite synthesis is associated with a release of hydrogen ions which must be excreted by the kidney The molar base/calcium ratio of 0 92 suggested by the hydroxyapatite structure (Table 1) is a little higher than that obtained by direct titration of dissolved bone mineral (21) presumably because of a different base/calcium ratio of the bone crystal surface layer and intercrystalline inorganic matter An estimate of 20 mEq of H released by the deposition of one gram of calcium in the growing skeleton is probably fairly accurate (10)

It should be appreciated that the effect of bone resolution (e g in chronic acidosis) on the NAB is much less predictably related to the calcium balance because apatite and non apatite components may be involved in varying proportions (5 13 20)

The effects of soft tissue growth on the NAB may be viewed in terms of contributions to both the output and the input The deposition of new body water can be conceived as a two step process whereby deposition of water (and

Table 1. *Variables of net acid balance in the growing infant*

| Source of net acid | Reaction | Directional contribution |
|---|---|---|
| *Body input* | | |
| Sulfuric acid production | HS—R(NH)—COOH ⟶ mCO + nH O + urea + SO | +2H |
| Intestinal absorption | ±UA ⟶ mCO + nH O | ±H |
| Urinary titratable OA | R—COOH ⟶ R—COO (urine) | +H |
| Parenteral therapy | ⟶ | ±H |
| *Body water input* | | |
| Skeletal growth | 10Ca + 4 8HPO + 1 2H PO + 2H O → (Ca (PO ) ) Ca(OH) | +9 2H |
| Soft tissue growth | ⟶ | ±H |
| *Output* | | |
| Renal net acid excretion | ⟶ | $\pm H^+$ |

The net contribution by the gastrointestinal tract is conveniently accounted for by measurement of dietary and fecal UA (see section on calculations). Taking the pK of orthophosphoric acid to be 6 8, a 4 1 mixture of equimolar solutions of secondary and primary phosphate will have a UA concentration of zero at pH 7 40 if a valence of −1 8 is assigned to phosphorus. Thus treating (total) P as a determined anion with the valence 1 8 allows phosphate in diet and stool to be quantitatively accounted for as titratable phosphate (Lennon et al. (14)).

It should be realised that whereas titration of the urine to a fixed end point at pH 7 40 ($P_{CO_2}$ = zero mm Hg, temperature 37 C) gives the correct estimate of the output of urinary titratable acid, because the production of an acid (or alkaline) urine in itself represents a loss (or gain) of net acid from the body, this principle does not apply to the titration of urinary organic acids. These should be titrated from their undissociated state (conventionally pH 2 7 (Van Slyke & Palmer (4)) to the pH of plasma at filtration. Moreover, since urinary titratable organic acid may include organic anions (base) absorbed from the intestine and subsequently excreted in a partly neutralized state, the direct use of total urinary titratable organic acid as an input item requires net intestinal absorption of undetermined anions (UA) to be included as a negative component of the input. Also, the urine may under some circumstances contain appreciable amounts of oxidizable acids such as lactic acid, which are still largely dissociated at pH 2 7. Finally, it may be pointed out that the measurement of total organic acid in the urine will include titratable non-metabolizable anions produced in the body and belonging conceptually to the sulfate category.

[a] For a discussion of soft tissue growth as an input variable, see p. 374.

neutral salts) leads to a slight dilution acidosis (p. 327) which in turn stimulates renal conservation of bicarbonate (an output effect). Deposition of soft tissue *solids* as an input variable may be evaluated by a *bioassay* in healthy infants (see p. 374).

The conceptual, theoretical and technical aspects of NAB measurement in growing infants will be considered in greater detail elsewhere (11). This report presents the results of studies of the mean daily NAB in ten *healthy* growing premature infants based on 70 complete 24-hour collections of urine and stool and daily formula analysis. The infants were fed modified cow's milk formulae (based on Similac preparations and evaporated milk) in amounts corresponding to about 120 Cal/kg/24 hours. Normal feeding schedules were not interfered with.

## METHODS

Complete 24-hour urine and stool specimens were collected for four to thirteen consecutive days by the method of Lin & Anderson (15). Stools (including contaminated diaper tissue) were dissolved in boiling concentrated nitric acid before measurement. The volumes of formula ingested were recorded and accurately timed with daily sample being obtained for analysis. Formula, urine and extracts of stool were analyzed for sodium and potassium by flame photometry, for calcium and magnesium by atomic absorption spectrophotometry, for chloride by the Buchler-Cotlove technique, and for (total) phosphorus by the method of Bagnall et al. (2). Urinary sulfate was determined by the method of Yatzidis et al. (28) and urinary titratable organic acid (OA) was estimated by titration between pH 2 70 and pH 7 60 (room temperature) following precipitation with Ca(OH), the values being corrected for titration of water as well as of creatinine (Table 1). Urinary net acid excretion was measured by titrating formaldehyde treated samples (9) to pH 7 40 (room temperature and $P_{CO_2}$ zero mm Hg). Blood acid base status (1) and body weight were determined daily.

such and the other representing net gains by the *body fluid* charging the kidney but not primarily affecting the total body content of acid or base To the first category belong contributions by oral intake (or vomiting) fecal excretion parenteral therapy endogenous production from neutral precursors (e g sulfuric acid originating by oxidation of sulfur-containing aminoacids) and titratable *non oxidizable* acid stranded in the body as a consequence of urinary loss of the conjugate anion of organic acid produced in the body To the second category belong titratable acid or base released in the body water as a result of ongoing skeletal mineralization or bone resolution respectively as well as any similar contributions to the net load on the kidney occurring by incorporation of phosphate residues into structural tissue proteins or lipids or by peptide bond formation and subsequent buffering of new protein It may be pointed out that such a distinction between retentional and distributional contributions and hence between balances of net acid in the body ( body balance ) and balances of net acid in the body water ( body water balance ) is to some extent arbitrary but it serves the useful function of separating irreversible processes (first category) from reversible processes (second category)

If net acid balances for extracellular fluid or for plasma were to be calculated redistribution of H between intra and extracellular water and between interstitial fluid and plasma respectively would have to be taken into account Also it should be recognized that changes in the net acid *concentration* of the plasma bringing about a renal response may occur at a net zero input i e in the absence of changes in the net acid *content* of any body water compartment This occurs characteristically with dilution acidosis and contraction alkalosis (Winters *et al* (27) Cannot *et al* (3)) see also p 323 Similarly changes in plasma $P_{CO_2}$ and in the plasma concentration of oxidizable organic acids may influence the renal net acid excretion and hence *indirectly* the NAB

## PRINCIPLES OF INPUT ASSAY

The various physiological processes which contribute directly to the NAB of the growing infant are summarized in Table 1 where $H^+$ and $-H$ in the righthand column denote gains of titratable acid and base respectively

Whereas measurement of the renal net acid excretion is relatively easy the independent quantitative characterization of the daily input poses formidable problems Within recent years studies by Relman *et al* (27) Lennon *et al* (14) have gone far toward resolving the problems of measurement of input in the non growing adult and valuable balance data have been gained (5 12 13) However experience with quantitative NAB measurements is still limited, and to our knowledge the NAB of the growing organism has never been approached in these terms In order for NAB data on growing infants to be interpretable the available techniques must be modified to provide specified quantitative information concerning any contribution to the balance arising from the processes of growth Thus changes in the net load of acid presented to the kidney due to deposition of base in the growing skeleton as well as any similar effect attributable to growth of the soft tissues must be evaluated

During the process of skeletal mineralization hydroxyapatite synthesis is associated with a release of hydrogen ions which must be excreted by the kidney The molar base/calcium ratio of 0 92 suggested by the hydroxyapatite structure (Table 1) is a little higher than that obtained by direct titration of dissolved bone mineral (21) presumably because of a different base/calcium ratio of the bone crystal surface layer and intercrystalline inorganic matter An estimate of 20 mEq of H released by the deposition of one gram of calcium in the growing skeleton is probably fairly accurate (10)

It should be appreciated that the effect of bone *resolution* (e g in chronic acidosis) on the NAB is much less predictably related to the calcium balance because apatite and non apatite components may be involved in varying proportions (5 13 20)

The effects of soft tissue growth on the NAB may be viewed in terms of contributions to both the output and the input The deposition of new body *water* can be conceived as a two-step process whereby deposition of water (and

Table 1 *Variables of net acid balance in the growing infant*

| Source of net acid | Reaction | Directional contribution |
|---|---|---|
| *Body input* | | |
| Sulfuric acid production | HS—R(NH )—COOH ⟶ mCO + nH$_2$O + urea + SO | +2H |
| Intestinal absorption | ±UA ⟶ mCO + nH O | ±H |
| Urinary titratable OA | R—COOH ⟶ R—COO (urine) | +H |
| Parenteral therapy | ⟶ | ±H |
| *Body water input* | | |
| Skeletal growth | 10Ca + 4 8HPO + 1 2H PO + 2H O → (Ca (PO ) ) Ca(OH)$_2$ | +9 7H |
| Soft tissue growth[b] | ⟶ | ±H |
| *Output* | | |
| Renal net acid excretion | ⟶ | ±H |

The net contribution by the gastrointestinal tract is conveniently accounted for by measurement of dietary and fecal UA (see section on calculations) Taking the pK of orthophosphoric acid to be 6 8 a 4 1 mixture of equimolar solutions of secondary and primary phosphate will have a UA concentration of zero at pH 7 40 if a valence of −1 8 is assigned to phosphorus Thus treating (total) P as a determined anion with the valence 1 8 allows phosphate in diet and stool to be quantitatively accounted for as titratable phosphate (Lemann *et al* (14))

It should be realized that whereas titration of the urine to a fixed end point at pH 7 40 (P o ~ zero mm Hg, temperature 37 C) gives the correct estimate of the output of urinary titratable acid because the production of an acid (or "alkaline ) alkaliferine in itself represents a loss (or gain) of net acid from the body this principle does not apply to the titration of urinary organic acids These should be titrated from their undissociated state (conventionally pH 2 7 cf Van Slyke & Palmer (24)) to the pH of plasma at filtration Moreover since urinary titratable organic acid may include organic anions (base) absorbed from the intestine and subsequently excreted in a partly neutralized state the direct use of total urinary titratable organic acid as an input item requires net intestinal absorption of undetermined anion (UA) to be included as a negative component of the input Also the urine may under some circumstances contain appreciable amounts of oxidizable acids such as lactic acid which are still largely dissociated at pH 2 7 Finally it may be pointed out that the measurement of total organic acid in the urine will include titratable non metabolizable anions produced in the body and belonging conceptually to the sulfate category

[b] For a discussion of soft tissue growth as an input variable see p 324

neutral salts) leads to a slight dilution acidosis (p 322) which in turn stimulates renal conservation of bicarbonate (an output effect) Deposition of soft tissue *solids* as an input variable may be evaluated by a *bioassay* in healthy infants (see p 324)

The conceptual theoretical and technical aspects of NAB measurement in growing infants will be considered in greater detail elsewhere (11) This report presents the results of studies of the mean daily NAB in ten healthy growing premature infants based on 70 complete 24 hour collections of urine and stool and daily formula analysis The infants were fed modified cow s milk formulae (based on Similac[2] preparations and evaporated milk) in amounts corresponding to about 120 Cal/kg/24 hours Normal feeding schedules were not interfered with

## METHODS

Complete 24 hour urine and stool specimens were collected for four to thirteen consecutive days by the method of Lui & Anderson (15) Stools (including contaminated diaper tissue) were dissolved in boiling concentrated nitric acid before measurement The volumes of formula ingested were recorded and accurately timed with daily samples being obtained for analysis Formula urine and extracts of stool were analyzed for sodium and potassium by flame photometry for calcium and magnesium by atomic absorption spectrophotometry for chloride by the Buchler Cotlove technique and for (total) phosphorus by the method of Bagnaki *et al* (2) Urinary sulfate was determined by the method of Yatzidis *et al* (28) and urinary titratable organic acid (OA) was estimated by titration between pH 2 70 and pH 7 60 (room temperature) following precipitation with Ca(OH)$_2$ the values being corrected for titration of water as well as of creatinine (Table 1) Urinary net acid excretion was measured by titrating formaldehyde treated samples (9) to pH 7 40 (room temperature and $P_{CO_2}$ ~ zero mm $H_g$) Blood acid base status (1) and body weight were determined daily

Table 2 *Results of analyses of formula stool and urine during seven day net acid balance study in a healthy premature infant (subject TO)*

| Day | FORMULA Volume (ml) | Na | K | Ca (mmol) | Mg | Cl | P | STOOL Na | K | Ca (mmol) | Mg | Cl | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 290 | 3 72 | 6 64 | 4 50 | 0 67 | 4 76 | 4 27 | 1 14 | 1 23 | 4 60 | 0 56 | 0 00 | 1 44 |
| 2 | 280 | 3 64 | 6 78 | 4 49 | 0 67 | 4 52 | 5 30 | 1 57 | 0 70 | 2 19 | 0 30 | 0 00 | 0 54 |
| 3 | 280 | 3 72 | 6 64 | 4 50 | 0 67 | 4 76 | 4 27 | 0 92 | 0 97 | 2 5[illegible] | 0 38 | 0 00 | [illegible] |
| 4 | 305 | 3 81 | 6 89 | 5 29 | 0 76 | 4 99 | 4 83 | 1 46 | 2 11 | 6 34 | 0 80 | 0 00 | 1 69 |
| 5 | 370 | 4 26 | 7 58 | 5 13 | 0 77 | 5 44 | 4 88 | 0 74 | 1 05 | 2 77 | 0 46 | 0 00 | [illegible] |
| 6 | 305 | 4 05 | 7 22 | 4 90 | 0 73 | 5 18 | 4 65 | 0 36 | 0 93 | 2 11 | 0 35 | 0 00 | [illegible] |
| 7 | 320 | 4 42 | 7 71 | 5 06 | 0 75 | 5 23 | 4 41 | 1 14 | 1 79 | 3 08 | 0 50 | 0 00 | [illegible] |

| Day | URINE Volume (ml) | Na | K | Ca (mmol) | Mg | Cl | P | Titr OA (mEq) | $SO_4^{--}$ (mmol) | TA (mEq) | NH (mEq) | Creatinine (mg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 135 | 2 54 | 3 91 | 0 02 | 0 01 | 4 08 | 0 75 | 2 14 | 0 07 | 1 32 | 2 00 | 23 09 |
| 2 | 170 | 1 67 | 4 59 | 0 02 | 0 01 | 2 84 | 1 65 | 2 53 | 0 03 | 1 68 | 3 81 | 24 89 |
| 3 | 115 | 1 03 | 3 33 | 0 01 | 0 00 | 2 03 | 1 69 | 1 89 | 0 08 | 1 17 | 1 45 | 19 25 |
| 4 | 135 | 0 92 | 3 29 | 0 02 | 0 01 | 1 79 | 1 60 | 2 21 | 0 09 | 1 32 | 1 29 | 17 63 |
| 5 | 130 | 1 30 | 3 30 | 0 04 | 0 02 | 2 12 | 1 61 | 2 09 | 0 13 | 1 54 | 1 63 | 18 97 |
| 6 | 153 | 1 65 | 4 43 | 0 03 | 0 01 | 2 56 | 1 71 | 2 45 | 0 18 | 1 70 | 1 84 | [illegible] 60 |
| 7 | 165 | 2 52 | 3 63 | 0 06 | 0 01 | 2 79 | 1 64 | 2 43 | 0 13 | 1 75 | 1 88 | [illegible] 13 |

For derivated NAB data see Table 3 Urinary TA is titrated to pH 7 60 (room temperature) and $P_{CO_2}$ = 0 mm Hg, see p 323

*Calculations* (values in mEq per 24 hours unless otherwise stated)

Body balance body input body output
Body input urinary sulfate + urinary OA − absorbed UA
Absorbed UA dietary UA fecal UA
UA (Na + K + Ca + Mg ) (Cl + 1 8 P (in mmol per 24 hours))
Body output renal net acid excretion (NAE)
H (H released by hydroxyapatite deposition) − calcium balance (mEq per 24 hours) 0 40 (Kildeberg (10))

The magnitude of any additional contributions to the balance (e g by soft tissue growth) not taken into account by the above measurements and calculations was estimated as follows In a healthy infant in a steady state it seems reasonable to assume that the kidney every day excretes the net load of acid presented to it This first approximation however requires two small corrections First slight changes in the balance associated with a small daily variation of the blood titratable base (blood base excess or BE) were approximated by calculating the corresponding gain of net acid as △ blood BE 0 5 body weight (in kg) Second the NAE was reduced by an amount corresponding to the daily retention of bicarbonate in new body water computed on the assumption that the average bicarbonate concentration of new (intra and extracellular) body water was 12 mEq per liter and that the quantity of new body water retained corresponded to 75 of the daily increase in body weight The resulting estimate of the daily net acid load was then compared to the measured body water input (urinary sulfate + urinary OA − absorbed UA + H c ) the difference representing the best estimate of residual unexplained (U fraction) contributions to the input

U fraction (NAE − △BE 0 5 body weight (kg) − 0 75 △body weight (kg) 12)−(urinary sulfate + urinary OA − absorbed UA + H c )

## RESULTS

Tables 2 and 3 provide details of measured and derived variables of the net acid balance in one healthy premature infant from the 12th to the 19th day of life The UA concentration of the milk preparation used (Similac[1]) was close to 30 mEq/l which resulted in a daily intake of about eight mEq of potential base (largely citrate) At the end of the seven day balance period the amount of UA ingested (61 3 mEq) was closely matched by a stool UA total of 58 1 mEq variations in the daily figures being apparently due to the fact that the (unmarked) stool specimens were not in any given 24 hour period precisely representative of the quantity of formula concurrently ingested It is note

Table 3. *Study of the balance of net acid from the 12th to the 19th day of life in a healthy premature infant (TO) weighing 1.4 kg*

(All values are in mEq per 24 hours)

| Day | $UA_{diet}$ | $UA_{stool}$ | $UA_{absorbed}$ | Urinary titratable OA | Urinary sulfate | $H_{Ca}$ | Net acid excretion | Body input | Body balance | U fraction |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 8.75 | 10.10 | −1.35 | 2.14 | 0.14 | −0.10 | 3.32 | 4.13 | 0.81 | −1.20 |
| 2 | 6.68 | 6.29 | 0.39 | 2.53 | 0.06 | 1.84 | 3.90 | 2.20 | −3.30 | 0.97 |
| 3 | 8.25 | 6.25 | 2.00 | 1.89 | 0.15 | 1.57 | 2.63 | 0.04 | −2.59 | 0.71 |
| 4 | 9.14 | 14.80 | −5.66 | 2.21 | 0.18 | −0.85 | 2.62 | 8.05 | 5.43 | −4.90 |
| 5 | 9.43 | 6.93 | 2.50 | 2.09 | 0.25 | 1.86 | 3.17 | 0.16 | −3.33 | 0.97 |
| 6 | 8.98 | 5.17 | 3.81 | 2.45 | 0.36 | 2.20 | 3.54 | −1.00 | −4.54 | 1.64 |
| 7 | 10.57 | 8.55 | 2.02 | 2.43 | 0.27 | 1.53 | 3.64 | 0.68 | −2.96 | 0.82 |
| Total | 61.30 | 58.09 | 3.21 | 15.74 | 1.41 | 8.05 | 24.42 | 13.94 | 10.48 | −0.99 |
| Average | 8.76 | 8.30 | 0.46 | 2.25 | 0.20 | 1.15 | 3.49 | 1.99 | −1.50 | −0.14 |

Diet Similac (Na 15 mmol/l K 25 mmol/l $Ca^{++}$ 17 mmol/l Mg 2.3 mmol/l Cl 17 mmol/l total P 16 mmol/l total S 7 mmol/l caloric value 680 Cal/l)

worthy that this net zero contribution of dietary intake and fecal excretion of base to the net acid input emerges as a difference between two large numbers. Table 3 shows that dietary UA intake and fecal UA excretion were in fact the two largest single variables of the NAB each being more than twice as large as the total NAI for the same period and much larger than any other single component of the balance. Because both measured (Na, K, Ca, Mg, Cl and phosphate) and unmeasured" (organic) ions of the dietary intake are *absorbable* these results suggest that fecal base excretion was actively regulated (see p. 327).

Columns 4 and 5 of Table 3 demonstrate that endogenous production of sulfuric acid accounted for an almost negligible fraction of the daily net acid input whereas the daily renal loss of titratable organic anion was considerable and remarkably constant. These findings are in marked contrast to values reported for adults on various diets where sulfate production is usually the most important determinant of the net acid input (8, 22). The average daily calcium balance was only +57 mg yet the estimated average contribution to the acid load presented to the kidney originating in the process of skeletal hydroxyapatite deposition amounted to 33 % of the average daily NAE [illegible] body NAB was negative accounting roughly for the rate of base deposition in skeleton and in new body water.

Mean daily values for all ten infants studied are presented in Table 4 which includes data on milk and stool calcium. It is seen that the same general pattern was observed in each case. Thus a surprisingly close relationship was demonstrated between mean daily values for dietary UA intake and fecal UA excretion, the overall mean rate of gastrointestinal UA absorption amounting to less than one mEq/day. An analysis of covariance applied to the 70 sets of paired individual $UA_{in}$ and $UA_{out}$ values obtained rejected significant interindividual differences in slope and intercept of linear regressions of $UA_{stool}$ on $UA_{diet}$ and produced the following weighted average of the individual regression parameters

$$UA_{stool} = 0.737\ UA_{diet} + 1.711 (p < 0.0005)$$

In seven of the ten infants studied the mean daily NAB was negative; in two, very low rates of calcium retention were associated with approximately zero NAB values, and in one (CL) a negative calcium balance compares with a NAB of +1.6 mEq. The significance of bone growth in determining the net acid balance of infants was established statistically by correlating individual mean daily values for $H^+_{Ca}$ and

Table 2 *Results of analyses of formula stool and urine during seven day net acid balance study i healthy premature infant (subject TO)*

| | FORMULA | | | | | | | STOOL | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Volume (ml) | Na | K | Ca (mmol) | Mg | Cl | P | Na | K | Ca (mmol) | Mg | Cl | P |
| 1 | 280 | 3 72 | 6 64 | 4 50 | 0 67 | 4 76 | 4 27 | 1 14 | 1 23 | 4 60 | 0 56 | 0 00 | [illegible] |
| 2 | 280 | 3 64 | 6 78 | 4 49 | 0 67 | 4 52 | 5 30 | 1 57 | 0 70 | 2 19 | 0 30 | 0 00 | [illegible] |
| 3 | 280 | 3 72 | 6 64 | 4 50 | 0 67 | 4 76 | 4 27 | 0 92 | 0 97 | 2 52 | 0 38 | 0 00 | [illegible] |
| 4 | 305 | 3 81 | 6 89 | 5 29 | 0 76 | 4 99 | 4 83 | 1 46 | 2 11 | 6 34 | 0 80 | 0 00 | [illegible] |
| 5 | 320 | 4 26 | 7 58 | 5 13 | 0 77 | 5 44 | 4 88 | 0 74 | 1 05 | 2 77 | 0 46 | 0 00 | [illegible] |
| 6 | 305 | 4 05 | 7 22 | 4 90 | 0 73 | 5 18 | 4 65 | 0 36 | 0 93 | 2 11 | 0 35 | 0 00 | [illegible] |
| 7 | 320 | 4 42 | 7 71 | 5 06 | 0 75 | 5 23 | 4 41 | 1 14 | 1 79 | 3 08 | 0 50 | 0 00 | [illegible] |

| | URINE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Volume (ml) | Na | K | Ca (mmol) | Mg | Cl | P | Titr OA (mEq) | $SO_4$— (mmol) | TA (mEq) | NH (mEq) | Creatinine (mg) |
| 1 | 135 | 2 54 | 3 91 | 0 02 | 0 01 | 4 08 | 0 75 | 2 14 | 0 07 | 1 32 | 2 00 | 23 09 |
| 2 | 170 | 1 67 | 4 59 | 0 02 | 0 01 | 2 84 | 1 65 | 2 53 | 0 03 | 1 68 | 3 81 | 24 89 |
| 3 | 115 | 1 03 | 3 33 | 0 01 | 0 00 | 2 03 | 1 69 | 1 89 | 0 08 | 1 17 | 1 45 | 19 75 |
| 4 | 135 | 0 92 | 3 29 | 0 02 | 0 01 | 1 79 | 1 60 | 2 21 | 0 09 | 1 32 | 1 29 | 17 63 |
| 5 | 130 | 1 30 | 3 30 | 0 04 | 0 02 | 2 12 | 1 61 | 2 09 | 0 13 | 1 54 | 1 63 | 18 92 |
| 6 | 153 | 1 65 | 4 43 | 0 03 | 0 01 | 2 56 | 1 71 | 2 45 | 0 18 | 1 70 | 1 84 | 22 60 |
| 7 | 165 | 2 52 | 3 63 | 0 06 | 0 01 | 2 79 | 1 64 | 2 43 | 0 13 | 1 75 | 1 88 | 24 13 |

For derivated NAB data see Table 3 Urinary TA is titrated to pH 7 60 (room temperature) and P -0 mm Hg see p 323

*Calculations* (values in mEq per 24 hours unless otherwise stated)

Body balance - body input body output
Body input - urinary sulfate + urinary OA - absorbed UA
Absorbed UA dietary UA - fecal UA
UA - (Na + K + Ca + Mg ) - (Cl + 1 8 P (in mmol per 24 hours))
Body output - renal net acid excretion (NAE)
$H_c^+$ (H released by hydroxyapatite deposition) - calcium balance (mEq per 24 hours) 0 40 (Kildeberg (10))

The magnitude of any additional contributions to the balance (e g by soft tissue growth) not taken into account by the above measurements and calculations was estimated as follows In a healthy infant in a steady state it seems reasonable to assume that the kidney every day excretes the net load of acid presented to it This first approximation however requires two small corrections First slight changes in the balance associated with a small daily variation of the blood titratable base (blood base excess or BE) were approximated by calculating the corresponding gain of net acid as Δ blood BE 0 5 body weight (in kg) Second the NAE was reduced by an amount corresponding to the daily retention of bicarbonate in new body water computed on the assumption that the average bicarbonate concentration of new (intra and extracellular) body water was 12 mEq per liter and that the quantity of new body water retained corresponded to 75 % of the daily increase in body weight The resulting estimate of the daily net acid load was then compared to the measured body val input (urinary sulfate + urinary OA - absorbed U + $H_c$ ) the difference representing the best estim of residual unexplained (U fraction) contribution the input

U fraction - (NAE - ΔBE 0 5 body weight (kg) 0 75 Δbody weight (kg) 12)-(urinary sulfat urinary OA - absorbed UA + $H_c$ )

## RESULTS

Tables 2 and 3 provide details of measured a derived variables of the net acid balance one healthy premature infant from the 12th the 19th day of life The UA concentration the milk preparation used (Similac®) was cl to 30 mEq/l which resulted in a daily inta of about eight mEq of potential base (larg citrate) At the end of the seven day balan period the amount of UA ingested (61 3 mE was closely matched by a stool UA total 58 1 mEq variations in the daily figures be apparently due to the fact that the (unmarke stool specimens were not in any given 24 ho period precisely representative of the quant of formula concurrently ingested It is not

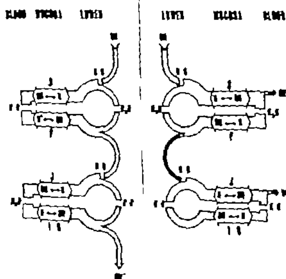


*Fig 1* Model of the transintestinal turnover of acid and base under conditions of (*a*) no net base absorption (i.e., equal rates of secretion of acid and base) and (*b*) net (complete) absorption of base (i.e., net luminal secretion of acid) *S P J I* and C stomach pancreas jejunum ileum and colon

be pointed out that any error resulting from this practice would tend to decrease unduly the value for the U fraction —and obscure the relation of the body NAB to the calcium balance

For some time the concept of homeostasis by growth originally developed by McCance & Widdowson (16) has dominated current views on the renal contribution to acid base homeostasis in the growing infant The present results suggest that the validity of this concept with respect to acid base metabolism be re-examined As far as skeletal growth and body water expansion are concerned growth appears to increase rather than reduce the demand for renal excretion of acid and moreover fecal base excretion may be an important factor

In the gastrointestinal tract very appreciable quantities of acid and base are subjected to a continuous turnover the rate of which may be several fold that of renal net acid excretion Although homeostatic mechanisms generally operate in close relation to sites of major or maximal transfer available information on gastrointestinal acid base metabolism appears not to have been examined with a view to disclosing specific homeostatic relationships between transintestinal acid base reactions and the acid base status of the body The results of the present study serve to raise this question

It is generally accepted that gastrointestinal absorption of bicarbonate occurs by a mechanism similar to that taking place in the renal tubules (luminal bicarbonate ions being neutralized by hydrogen ions secreted (by ion exchange) into the lumen with an equivalent amount of bicarbonate being delivered to the venous return (6 17 19 26) Thus substantial amounts of bicarbonate may be absorbed by the stomach additional bicarbonate ions (secreted by the pancreas) being absorbed by jejunal H secretion (17 25) Accepting this mechanism of absorption permits the important deduction that if the *total* secretion of acid (by stomach and jejunum) and the total secretion of base (by pancreas ileum and colon) were maintained at *equal* rates net bicarbonate absorption would be impossible ingested bicarbonate (or OH ) being shunted through the gastrointestinal tract (Fig 1 *a*) On other hand net absorption of any given quantity of bicar

Table 4 *Mean daily values (mEq per 24 hours) for components of the balance of net acid in ten healthy premature infants*

| Subject | Weight on day 1 (kg) | Number of days studied | $UA_{int}$ | $UA_{fec}$ | $UA_{absorbed}$ | $Ca_{int}$ | $Ca_{bal}$ | Urinary titratable OA | Urinary sulfate | $H_c^+$ | Net acid excretion | Body input | Body balance | U fraction |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TO | 1.41 | 7 | 8.76 | 8.30 | 0.46 | 9.67 | 6.75 | 2.25 | 0.20 | 1.15 | 3.49 | 1.99 | −1.50 | 0.14 |
| SM | 1.61 | 7 | 6.16 | 5.70 | 0.45 | 5.73 | 3.61 | 2.65 | 0.05 | 1.03 | 3.37 | 2.25 | −1.12 | 0.13 |
| NE | 1.16 | 6 | *6.23* | *6.61* | *−0.38* | *6.79* | *5.86* | *2.07* | *0.21* | *0.35* | *2.63* | *2.66* | *0.03* | *−1.[illegible]* |
| AT | 1.96 | 4 | 6.92 | 6.60 | 0.32 | 7.09 | 5.62 | 2.66 | 0.27 | 0.57 | 3.08 | 2.61 | −0.47 | 0.[illegible] |
| RO | 1.85 | 13 | 13.91 | 12.34 | 1.58 | 14.02 | 9.75 | 2.70 | 1.54 | 1.62 | 4.11 | 2.66 | −1.45 | 0.13 |
| SZ | 1.32 | 7 | 8.99 | 7.19 | 1.81 | 8.64 | 6.06 | 2.64 | 0.47 | 0.90 | 3.19 | 1.30 | −1.89 | 1.[illegible] |
| CL | 1.35 | 8 | 6.26 | 7.05 | −0.79 | 4.47 | 5.08 | 2.02 | 0.14 | −0.27 | 1.35 | 2.95 | 1.60 | 1.[illegible] |
| ST | 1.82 | 4 | 4.99 | 4.98 | 0.01 | 3.72 | 2.69 | 1.37 | 0.32 | 0.40 | 1.49 | 1.68 | 0.19 | 1.[illegible] |
| BR | 1.58 | 7 | 10.91 | 9.48 | 1.43 | 11.46 | 7.70 | 2.36 | 0.23 | 1.44 | 4.00 | 1.16 | −2.84 | 0.[illegible] |
| HO | 1.53 | 7 | 8.05 | 5.97 | 2.08 | 4.10 | 2.96 | 1.70 | 0.04 | 0.40 | 1.76 | −0.34 | −2.10 | 1.[illegible] |
| Average | | | 8.12 | 7.42 | 0.70 | 7.57 | 5.61 | 2.24 | 0.35 | 0.76 | 2.85 | 1.89 | −0.96 | −0.4 |

body NAB.[1] An analysis of linear regression based on the data of Table 4 gave the following result: NAB = 1.76 $H_{Ca}$ + 0.38 ($r$ = 0.77, $p < 0.01$ for eight degrees of freedom). Considering the sum of $H_{Ca}$ and the estimated rate of bicarbonate deposition in new body water rather than $H_{Ca}$ alone resulted in slight improvement of the correlation ($r$ = 0.80).

The residual fraction of the input (Table 4, column 14) was established on the assumption that the NAE corrected for estimated changes in body base represents the net load of acid actually presented to the kidney (p. 324). Calculating the body balance as described in the section on methods defines the residual contribution as distributional. Such classification, however, is to some extent arbitrary and the U fraction may, in addition to the effect of propagated analytical error inherent in the large number of measurements required in order to derive the balance (11), include several independent variables such as phosphate ester formation and synthesis and subsequent buffering of proteins in general. The U fraction was found to be very small, however, with an overall daily mean value of only −0.3 mEq. Probably, therefore, any contribution of net acid to the water phase originating in the deposition of new soft tissue solids is small enough to be disregarded in the evaluation of the daily net acid balance.

## DISCUSSION

It appears from the present data that the pattern of net acid input displayed by the growing infant differs greatly from that seen in the adult. The growth of protein stores appears to limit sulfate production (for any given sulfur intake, see Table 3) and the impressive rate of excretion of titratable OA by the infant establishes renal loss of organic anion as a major source of net acid at this age. Also the close correlation between the daily calcium balance and the NAB bears out the impact on the latter represented by skeletal growth.

The residual component of the input, designated as the U fraction, has been tentatively relegated to soft tissue growth (apart from body water expansion). Titration of urinary OA was carried out routinely to an endpoint a little above the average plasma pH of healthy premature infants (see note to Table 1). It may

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[1] In this case the more sensitive approach of analysis of covariance is not feasible because daily discrepancies between dietary intake and stool output would heavily bias the result in favour of (negative) correlation.

The opposite is true for the analysis of the relationship of fecal UA excretion to dietary UA intake described in the foregoing.

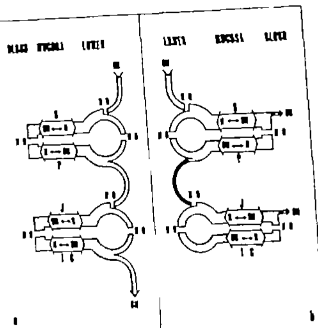


*Fig 1* Model of the transintestinal turnover of acid and base under conditions of (*a*) no net base absorption (i e equal rates of secretion of acid and base) and (*b*) net (complete) absorption of base (i e net luminal secretion of acid) *S P J I* and *C* stomach pancreas jejunum ileum and colon

be pointed out that any error resulting from this practice would tend to decrease unduly the value for the U fraction—and obscure the relation of the body NAB to the calcium balance

For some time the concept of homeostasis by growth originally developed by McCance & Widdowson (16) has dominated current views on the renal contribution to acid base homeostasis in the growing infant The present results suggest that the validity of this concept with respect to acid base metabolism be re-examined As far as skeletal growth and body water expansion are concerned growth appears to increase rather than reduce the demand for renal excretion of acid and moreover fecal base excretion may be an important factor

In the gastrointestinal tract, very appreciable quantities of acid and base are subjected to a continuous turnover the rate of which may be several fold that of renal net acid excretion Although homeostatic mechanisms generally operate in close relation to sites of major or maximal transfer available information on gastrointestinal acid base metabolism appears not to have been examined with a view to disclosing specific homeostatic relationships between "transintestinal" acid base reactions and the acid base status of the body The results of the present study serve to raise this question

It is generally accepted that gastrointestinal absorption of bicarbonate occurs by a mechanism similar to that taking place in the renal tubule luminal bicarbonate ions being neutralized by hydrogen ions secreted (by ion exchange) into the lumen with an equivalent amount of bicarbonate being delivered to the venous return (6 17 19 26) Thus substantial amounts of bicarbonate may be absorbed by the stomach additional bicarbonate ions (secreted by the pancreas) being absorbed by jejunal H secretion (17 25) Accepting this mechanism of absorption permits the important deduction that if the total secretion of acid (by stomach and jejunum) and the total secretion of base (by pancreas ileum and colon) were maintained at *equal* rates net bicarbonate absorption would be impossible ingested bicarbonate (or OH ) being shunted through the gastrointestinal tract (Fig 1 *a*) On other hand net absorption of any given quantity of bicar

bonate requires an equivalent amount of acid to be *secreted in excess of ongoing base secretion* at other levels of the gastrointestinal tract Bicarbonate absorption, therefore requires an *adjustment* of the relative rates of gastrointestinal $H^+$ and $OH^-$ secretion (Fig 1 *b*) This argument holds immediately for bicarbonate (and $OH^-$) ions only with one additional assumption being required in order to allow non bicarbonate UA to be considered in the same general terms—viz that the conjugate buffer acids (arising by $H^+$ donation to non bicarbonate UA) comply with the principle of absorption by non ionic diffusion With this assumption the model depicted in Fig 1 would apply equally well to organic anions in the diet Actually the available evidence indicates that organic acids (and bases) do penetrate the gastrointestinal membrane by diffusion of the (fat soluble) uncharged moiety (7 23 25)

If following absorption the buffer acid in question were to be oxidized in the tissues the resulting net acid impact on the body would be registered as absorbed UA To the extent that the absorbed acid were subsequently excreted as anion in the urine an additional contribution to the net acid input would result Such effect however has nothing to do with the gastrointestinal handling of the substance—and would be quantitatively accounted for in the balance by titration of urinary OA

*It appears that in the healthy growing premature infant this intestinal base shunt allows ingested base (dietary UA) to be quantitatively excreted in the stool (fecal UA)* (If this did not occur oxidation of the amount of UA contained in the daily diet would probably suffice to produce an alkaline urine!) Given the magnitude of the gastrointestinal transport of base it is easy to imagine that even a modest disproportion between the rates of intake and loss at this level might result in a net load far exceeding that which is normally presented to the infantile kidney This occurs characteristically in infantile diarrhea but certain other clinical acid base disturbances e g 'congenital alkalosis' (4 18) and 'postoperative metabolic alkalosis' (10) may in a more restricted sense represent disorders of the transintestinal acid base homeostasis Accepting the view of a specifically regulated fecal excretion of base as proposed by our model raises the question of the nature of the direct stimulus to which the regulating mechanism responds Answers to this question at the moment must remain speculative, but both the luminal and the arterial acid base status may be involved

Conceivably continued work along the lines suggested in the present report may open a new investigative field with important implications for growth physiology and nutrition as well as for the study of infantile renal function The data indicate that in the evaluation of the acid base homeostasis of the infant more emphasis should be placed on the role of the gastrointestinal tract Furthermore quantitative information of the relative magnitudes of distributional dilutional and retentional components of several common clinical acid base disturbances would greatly improve our understanding of their development in sick infants and facilitate proper management

## SUMMARY

A method for the quantitative assessment of the balance of net acid (NAB) in growing infants with a changing body composition is described Results of seventy measurements of the daily NAB in healthy growing premature infants ingesting modified cow s milk formulae are reported The relative contributions of the various determinants of the daily net acid input in the infant differ from those in the normal adult and growth appears to be associated with negative net acid balances due to base deposition in skeleton and new body water The daily load of undetermined anion (UA) in the diet was found to be the largest single component of the NAB Moreover a strong correlation between the rates of dietary UA intake and fecal UA excretion suggested an active regulation of the transintestinal acid base balance A model for such regulation based upon available evidence concerning the transport of

acid and base across the gastrointestinal membrane is proposed and briefly discussed


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# IDENTICAL SYNDROMES OF CEREBRAL PALSY IN THE SAME FAMILY

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Fortunately cerebral palsy (CP) syndromes only infrequently appear in more than one member of a family Identical syndromes found in two or more siblings are even more rare but they are of interest from the geneti cal point of view Experiences of one of the authors (B H) from the cerebral palsy clinic in Uppsala during the years 1956–67 suggest ed more familial cases of congenital ataxia and ataxic diplegia within this last mentioned group than would be expected by chance The main objects of the present investigation were to collect data for an analysis of familial cases of cerebral palsy in order to distinguish clini co genetic entities and study their prevalence probable mode of inheritance and clinical char acteristics

The family investigations were not only con fined to first degree relatives but also included grandparents aunts uncles first cousins and relations of the same degree However the in quiries sent out were directed particularly to families in which more than one sibling had cerebral palsy

*Cerebral palsy* was defined according to the criteria given at the Little Club meeting in Ox ford 1959 (28) and Edinburgh 1964 (7) We want to stress that according to these criteria syndromes designated as cerebral palsy are non progressive This ruled out disorders such as the different heredodegenerative ataxias the hereditary spastic paraplegias of the progres sive type and the Sjogren Larsson disease ( The progressive spastic paraplegias of the ... ligmuller Strumpell Lorrain type (1 37) espe cially can be difficult to differentiate from spastic paraplegias of the cerebral palsy group because of their usually very slow progression

Not a few authors discuss cerebral palsy without distinguishing between progressive and non progressive disorders (6 33) In the great majority of our cases, however the stationary course was confirmed by repeated observations over a number of years at the same centre of ten by the same doctor In the remaining cases we obtained reliable information from parents or other sources which assured us that with all probability the condition was non progres sive

*Classification* There is no uniform classifi cation system of the different types of cerebral palsy The classification used by the present authors is shown in Table 1 The different terms used will be defined below

## CLINICAL MATERIAL

The investigation included both children and adults All families with more than one member having a cerebral palsy syndrome were sought in two ways Firstly inquiries were sent to all cerebral palsy clin ics regional cerebral palsy institutions central county registers for mentally retarded and institu tions and schools for mentally retarded over the whole of Sweden In all 218 such inquiries were sent Secondly inquiries were distributed to all fa

Table 1 *The approximate present distribution of the different cerebral palsy syndromes in children according to the classification mainly used in Sweden*

| | |
|---|---|
| Spastic syndromes | |
| Hemiplegia | 25 |
| Diplegia | 30 |
| Tetraplegia | 5 |
| Approx distrib | 50–60 |
| Dyskinetic syndromes | |
| Mainly athetotic | 5 |
| Mainly dystonic | 20–25 |
| Approx distrib | 25–30 |
| Ataxic syndromes | |
| Congenital ataxia | 5–7 |
| Ataxic diplegia | 5–7 |
| Approx distrib | 10–15 |

milies belonging to the Swedish Parents Association for Cerebral Palsy (2561 inquiries) See Table 2

Forty three families were collected in this way. Due to the present complete registration of cerebral palsy and mental subnormality in the younger age groups in Sweden this material probably covered practically all relevant cases below 19 years of age in the whole country However it was probably not quite representative for older age groups particularly not for adults with normal intelligence Furthermore there may have been a certain overrepresentation of families with more than one affected first degree relative compared to families with affected members of more distant relationships owing to the method of sampling

Table 2 *Distribution of inquiries and answers*

| | No of inquiries | No of families with more than one member reported |
|---|---|---|
| Families belonging to Swedish Parents Association for Cerebral Palsy | 2561 | 39 |
| Central county registers for mentally retarded | 23 | 5 |
| Institutions and clinics for cerebral palsy | 33 | 32 |
| Institutions and schools for mentally retarded children and adults | 16 | 16 |

Some cases were reported from more than one source Altogether 3 triplicate and 6 duplicate answers were received

Table 3 *Number of families in which more than one member was found to have a cerebral palsy syndrome*

| | No of families | No of cases |
|---|---|---|
| Identical syndromes with normal perinatal history | 16 | 43 |
| Identical syndromes with abnormal perinatal history | 3 | 6 |
| Non identical syndromes | 24 | 49 |
| Total number | 43 | 98 |

The material was divided into three different groups

1 Families with clinically identical CP syndromes and a history of normal pregnancy delivery and neonatal period

2 Families with clinically identical CP syndromes and an abnormal perinatal history

3 Families with non identical forms of CP syndromes

All families in the group with identical forms and normal perinatal histories were visited A thorough family history was taken and all patients except for two who did not allow a follow up examination were examined neurologically by one of the authors (G S) A standard case record form was used In addition metabolic screening was performed on a 12 hour sample of urine with a battery of tape and tube tests and including also two-dimensional paper chromatography for amino acid determination Chromosome analysis of cultured peripheral blood cells was made on at least one of the affected members in every family by one of the authors (K H G)

## RESULTS AND DISCUSSION

The material consisted of 43 families in which more than one member was affected with cerebral palsy In 30 of these families the affected cases were siblings The distribution of the families and patients into the non identical and the two identical groups is shown in Table 3

The figures given in Table 3 for the identical syndromes with an abnormal perinatal history refer to two families each with two siblings with an identical form of dystonia combined with athetosis caused by kernicterus Further one pair of dizygotic twins both with

This part of the examination was kindly performed by Assistant Professor Leif Hambraeus the Perinatal Laboratory University Hospital of Uppsala

Table 4 *Distribution of families and patients in the three groups according to type of cerebral p[illegible] syndrome*

| | Identical familial cases with normal perinatal history | | Identical familial cases with abnormal perinatal history | | Non identical fa[illegible] cases distributed among 74 familie[illegible] |
|---|---|---|---|---|---|
| | No of families | No of cases | No of families | No of cases | No of cases |
| Spastic syndromes | | | | | |
| Hemiplegia | | | | | 15 |
| Diplegia | 1 | 2 | 1 | 2 | 10 |
| Tetraplegia | 1 | 2 | | | 5 |
| Dyskinetic syndromes | | | | | |
| Mainly athetotic | | | | | 2 |
| Mainly dystonic | 1 | 2 | 2 | 4 | 3 |
| Ataxic syndromes | | | | | |
| Congenital ataxia | 10 | 25 | | | 2 |
| Ataxic diplegia | 3 | 12 | | | 7* |
| Type of CP uncertain | | | | | 7 |
| Total number | 16 | 43 | 3 | 6 | 49 |

This group included 3 cases with infantile hydrocephalus (18)

spastic diplegia, were born prematurely and had had serious asphyctic periods and anaemia in the neonatal period which probably was the main cause of their cerebral palsy

For the more common types of cerebral palsy the non identical familial cases showed an approximately similar distribution among the different syndromes as might be expected for an unselected clinical material (cf Tables 1 and 4) Concerning the more rare types of cerebral palsy the number of cases were too few to be analysed in this respect

The remainder of this paper will be confined to the group consisting of identical cases of cerebral palsy within the same family where the pregnancy and perinatal history were normal There was evidently an excess of ataxic forms in this group (cf Table 4) since ataxia and ataxic diplegia are otherwise found in only 12–13 per cent of the cases in unselected series of cerebral palsy (5 21)

The chromosome studies and the laboratory screening tests in our patients revealed no abnormalities of aetiological significance The familial material of probably inherited types of cerebral palsy will be discussed further under each sub group below

*Congenital ataxia*

Congenital ataxia was defined accordin[illegible] Ingram (22) It means briefly a pure and n[illegible] progressive cerebellar ataxia without any [illegible]ramidal signs

Our material contained 10 families in wh[illegible] 2–3 siblings had a non progressive congen[illegible] ataxia The age of the patients varied in [illegible] range of 2–41 years 10 patients were less th[illegible] 19 years of age The family data of the [illegible]tients are summarized in Table 5 Except [illegible] the father in family 1 with congenital blindn[illegible] probably due to toxoplasmosis and the mot[illegible] of EA in family 9 with a psychosis, none [illegible] the parents had a neurological or mental d[illegible]ease or was mentally retarded

Consanguinity was found in three famil[illegible] the parents being first cousins in one fam[illegible] and third cousins in two families In one [illegible]mily also a first cousin of two affected si[illegible]lings was similarly affected

In all patients except one (case AG, Table 5) there was moderate (on educable le[illegible]el) to severe (on non educable level) men[illegible] retardation Many of the older children a[illegible] the adults had well compensated for their cer[illegible]bellar dysfunction with increasing age Th[illegible]

Table 5. *Some data of the patients with congenital ataxia*

| Family No | Case Notation | Sex | Age yrs | Relationship | Order in sibship | Consanguinity | Degree of mental retardation | Additional hereditary data |
|---|---|---|---|---|---|---|---|---|
| 1 | KB | ♂ | 18 | Siblings | 2/4 | 0 | Severe | Father cong retinal atrophy (Toxoplasmosis?) |
| | BB | | 11 | | 4/4 | | Severe | |
| | AE | ♀ | 2 | Monozygotic twins | 2/3 | 0 | Severe (?) | |
| | FE | ♀ | 2 | | 3/3 | | Severe | |
| 3 | RG | | 22 | | 3/7 | 0 | Severe | 2 of the mother's siblings died in infancy both had seizures 2 of the maternal grandmother's sister's children were mentally retarded |
| | MG | | 17 | Siblings | 5/7 | | Severe | |
| | GG | ♀ | 14 | | 6/7 | | Severe | |
| 4 | LG | | 25 | Siblings | 3/5 | Parents third cousins | Moderate | 1/5 died at delivery |
| | AG | ♀ | 23 | | 4/5 | | None | |
| 5 | KH | ♂ | 10 | Siblings | 1/3 | 0 | Severe | |
| | MH | ♂ | 2 | | 3/3 | | Severe | |
| 6 | MJ | | 7 | Siblings | 1/3 | 0 | Moderate | First cousin congenital heart disease |
| | BJ | ♀ | 4 | | 2/3 | | | |
| 7 | BP | ♂ | 35 | | 1/9 | 0 | Severe | Brother (5/9) suspected phenylketonuria Second cousin mentally retarded |
| | AP | | 36 | Siblings | 2/9 | | Severe | |
| | GP | | 24 | | 6/9 | | Severe | |
| 8 | KS | | 41 | | 2/4 | 0 | Severe | |
| | SS | | 40 | Siblings | 3/4 | | Severe | |
| | AS | ♂ | 37 | | 4/4 | | Severe | |
| 9 | US | | 24 | Siblings | 1/11 | 0 | Severe | Maternal grandmother of US epilepsy Uncle of US and mother of EA psychosis 2 brothers of EA mentally retarded |
| | MS | | 21 | | 3/11 | Parents third cousins | Severe | |
| | EA | | 29 | First cousin of MS and US | 6/10 | | Moderate | |
| 10 | MT | | 41 | | 2/4 | Parents first cousins | Moderate | |
| | TT | ♂ | 27 | Siblings | 3/4 | | Moderate | |
| | IT | | 3 | | 4/4 | | Moderate | |

Moderate mental retardation educable Severe mental retardation non-educable

was particularly true for their disturbance of coordination of the movements in the upper limbs. This characteristic trait of the natural history of congenital non progressive cerebellar ataxia has been observed earlier by several authors (22 31 36)

In one of the families (No 4 in Table 5) the father was physician. The parents were third cousins. One daughter 26 years old was moderately mentally retarded had a hearing defect and showed as a child an evident cerebellar ataxia with marked intention tremor one of the differential diagnoses being Friedreich's ataxia. With increasing age she had well compensated for her disturbance of co-ordination. The main finding at the follow up examination was moderate [illegible] retardation slight dyskine[illegible] from her history and her father's continuous observations we were prone to classify her condition as a true congenital ataxia. A younger sister of this patient (whom we were not personally permitted to examine) was according to her father slightly ataxic but mentally normal and had some dysarthria and a hearing defect. We considered her disorder to be a less pronounced form of the same type of ataxia found in her older sister.

Two brothers (family No 5) had besides ataxia a small schisis of the occiput in direct continuation with foramen magnum and one of these brothers also had a cleft palate. Pneumoencephalographies had not been performed in these two cases but it is of interest to note that in the literature several of the reported cases with agenesis of the cerebellar

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These clinical data were kindly placed at our disposal by Dr H Voss Dept of Pediatrics County Hospital Sandviken

vermis also had a schistasis of the occiput (29) These findings together with the observation made by de Haene (17) that one of three siblings with congenital cerebellar ataxia was found at autopsy to have agenesis of the cerebellar vermis suggest one possible pathogenetic explanation for at least some of the congenital ataxic syndromes However in contrast to our patients the courses of the patients reported by de Haene were stated to be progressive the affected siblings all died before the age of 8 years of different intercurrent diseases Their clinical courses were however not consistent with the findings at autopsy which showed a brain with no sign of degeneration

One patient (KS in family 8) had one of her legs amputated just below the knee at 1 / years of age because of a serious infection At this time she had not been able to walk and later on she was unable to learn to use a prosthesis because of great problems with her balance Her two affected siblings exhibited mental retardation and an ataxia predominating in the legs Although it was impossible to evaluate her gait we considered that with all probability patient KS suffered from the same disease as her siblings this patient also showing mental retardation slight ataxia in the arms and dysarthria

The findings in our material of identical syndromes among siblings both sexes affected apparently healthy parents and consanguinity in at least three of the families suggest an autosomally recessive mode of inheritance for the great majority of familial cases of congenital ataxia

Of the cases of congenital ataxia in siblings reported in the literature (17 22 24 25 27 30 34 36 42 43) all have been clinically non progressive except the above mentioned patients of de Haene (17) Mental retardation usually of severe degree was an almost consistent co sign However Schutt (36) reported two siblings with congenital ataxia and normal intellectual development In all the families mentioned above the probable mode of inheritance was simple autosomal recessive Adler (2) however in his investigation of familial cerebral palsy reported a family with non progressive congenital ataxia combined with slight mental retardation in three generations The mode of inheritance was not discussed in this paper but as more than one generation and both sexes were affected and as there was no consanguinity of parents a simple dominant form seems most probable

The causes of congenital ataxia have mainly been considered to be of prenatal origin (7) Gross malformations of the cerebellum e agenesis of the cerebellar vermis have alread been mentioned Another pathological entity early familial cerebellar degeneration d scribed by Norman (31) and Jervis (24 h However Lamy *et al* (27) consider this entit to be not an early degenerative process but histologically defined cerebellar dysgenesis, hypothesis also discussed by Norman (30)

Few chromosome studies in cases of co genital ataxia have been reported No abno malities have been revealed hitherto whi well consistent with the normal karyotyp found in our cases

Inborn errors of metabolism tend to g progressive clinical pictures at least during t early stages of the disease It is therefore n surprising that the metabolic screening tests our patients with congenital ataxia did not r veal any abnormalities of pathogenetic intere The only abnormal finding among our ca was in a family where four siblings were me tally retarded at the imbecile level Three them had cerebellar ataxia and a normal u nary amino acid chromatogram The four who exhibited severe generalized rigidity h and increased phenylalanine excretion in t urine The biochemical analyses of this pati are not yet completed and the findings are d ficult to evaluate but probably they have connection with the congenital ataxias of siblings

The percentage of genetically determin forms of congenital ataxia associated with m derate or severe mental retardation can be c culated roughly as follows

Exact figures for all individuals with ce bral palsy are still lacking in Sweden but number of children and teenagers below year of age in 1960 has been calculated to 3150 (40) Approximately 6 per cent or 1 of these patients should have an ataxia acco ing to the frequency distribution in the larg Swedish material (5) One third (63 of the should be moderately or severely mentally tarded (22) With 10 patients in our mater

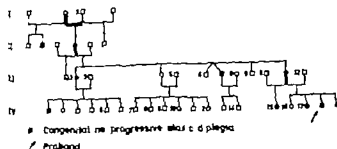



Fig 1 Pedigree showing congenital nonprogressive ataxic diplegia in three generations

within this group and less than 19 years of age a minimum figure for the proportion of familial forms of congenital ataxias with moderate or severe mental retardation will be 16

Since the number of children per family in Sweden is usually small most cases of autosomal recessive hereditary diseases occur sporadically During the last two decades the number of children per fertile marriage has been between two and three and the percentage of isolated non familial autosomal recessive cases should be approximately two thirds of all cases with that mode of inheritance Thus the proportion of inherited forms of congenital ataxia with mental retardation is even higher or approximately 50 per cent This figure means that if a child is born who proves to be mentally retarded and to have a congenital cerebellar ataxia we must be aware of the risk that the condition may be genetically determined particularly if the parents are related

### *Ataxic diplegia*

Ataxic diplegia was defined according to Ingram (22) meaning a cerebellar ataxia combined with spastic pareses mainly of the legs

At the physical examination nothing was found that could separate the hereditary case from others with a different etiology

We found three families in two families two siblings had an identical form of congenital non progressive ataxic diplegia A 16 year old girl and her 12 year old brother both high grade mental defectives and with healthy parent have been reported previously by Bille & Hagberg (8) The other two siblings a boy and a girl had in addition to congenital non progressive ataxic diplegia a cellular immunological defect proved for the girl (20) and highly suspected for the boy These siblings showed subnormal intellectual development In neither of these two families was any consanguinity found and the parents were healthy

The third family was of particular interest since it revealed members affected with ataxic diplegia in at least three generations The pedigree is shown in Figure 1

Our proband (IV 18) was a boy born in 1959 His sister (IV 19) born in 1960 was also affected Both children had been followed at our clinic for three years and no neurological progress had been observed On the contrary a steady functional improvement was noted in these two siblings The father of the proband and his relatives were healthy

The mother 41 years old (III 11) had a typical ataxic diplegia the upper limbs were only very little affected She was unable to walk without support before the age of 10 years and her clinical course was characterized by steady functional improvement Her older brother (III 7) had an almost identical clinical course H also had a twin brother (III 6) who died at 8 months of age The cause of death was probably spasmophilia

An elder sister of the proband's mother (III ) first walked without support when she was three years old At 9 years of age she was found to have an equinus deformity of the feet spastic involvement of the lower limbs and disturbance of coordination indicating ataxia at least in the legs Now 45 years old she had only very little trouble with slight spasticity in her legs and a minor cerebellar ataxia predominating in her legs

The maternal grandmother of the proband (II 4) was said to have had weak legs and feet as a child but later had almost normal leg function She died at 75 years of age from a heart disease

Her younger brother (II 2) still living at the age of 77 years had as a child the same gait trouble as his sister He was not able to walk without support until he was 4 years of age His clinical course was characterized by a steady functional improvement

vermis also had a schistasis of the occiput (29) These findings together with the observation made by de Haene (17) that one of three siblings with congenital cerebellar ataxia was found at autopsy to have agenesis of the cerebellar vermis suggest one possible pathogenetic explanation for at least some of the congenital ataxic syndromes However in contrast to our patients the courses of the patients reported by de Haene were stated to be progressive the affected siblings all died before the age of 8 years of different intercurrent diseases Their clinical courses were however not consistent with the findings at autopsy which showed a brain with no sign of degeneration

One patient (KS in family 8) had one of her legs amputated just below the knee at 1½ years of age because of a serious infection At this time she had not been able to walk and later on she was unable to learn to use a prosthesis because of great problems with her balance Her two affected siblings exhibited mental retardation and an ataxia predominating in the legs Although it was impossible to evaluate her gait we considered that with all probability patient KS suffered from the same disease as her siblings this patient also showing mental retardation slight ataxia in the arms and dysarthria

The findings in our material of identical syndromes among siblings both sexes affected, apparently healthy parents and consanguinity in at least three of the families suggest an autosomally recessive mode of inheritance for the great majority of familial case of congenital ataxia

Of the cases of congenital ataxia in siblings reported in the literature (17 22 24 25 27 30 34 36 42 43) all have been clinically non progressive except the above mentioned patients of de Haene (17) Mental retardation usually of severe degree was an almost consistent co sign However Schutt (36) reported two sibling with congenital ataxia and normal intellectual development In all the families mentioned above the probable mode of inheritance wa simple autosomal recessive Adler (2) however in his investigation of familial cerebral palsy reported a family with non progressive congenital ataxia combined with slight mental retardation in three generations The mode of inheritance was not discussed in this paper but as more than one generation and both sexes were affected and as there was no consanguinity of parents, a simple dominant form seems most probable

The causes of congenital ataxia have mainly been considered to be of prenatal origin (? Gross malformations of the cerebellum e agenesis of the cerebellar vermis have already been mentioned Another pathological entity is early familial cerebellar degeneration described by Norman (31) and Jervis (24 25) However Lamy *et al* (27) consider this entity to be not an early degenerative process but a histologically defined cerebellar dysgenesis a hypothesis also discussed by Norman (30)

Few chromosome studies in cases of congenital ataxia have been reported No abnormalities have been revealed hitherto which is well consistent with the normal karyotypes found in our cases

Inborn errors of metabolism tend to give progressive clinical pictures at least during the early stages of the disease It is therefore not surprising that the metabolic screening tests in our patients with congenital ataxia did not reveal any abnormalities of pathogenetic interest The only abnormal finding among our cases was in a family where four siblings were mentally retarded at the imbecile level Three of them had cerebellar ataxia and a normal urinary amino acid chromatogram The fourth who exhibited severe generalized rigidity had and increased phenylalanine excretion in the urine The biochemical analyses of this patient are not yet completed and the findings are difficult to evaluate but probably they have no connection with the congenital ataxias of the siblings

The percentage of genetically determined forms of congenital ataxia associated with moderate or severe mental retardation can be calculated roughly as follows

Exact figures for all individuals with cerebral palsy are still lacking in Sweden but the number of children and teenagers below 1? year of age in 1960 has been calculated to be 3150 (40) Approximately 6 per cent or 18? of these patients should have an ataxia according to the frequency distribution in the largest Swedish material (5) One third (63 of them should be moderately or severely mentally retarded (22) With 10 patients in our material

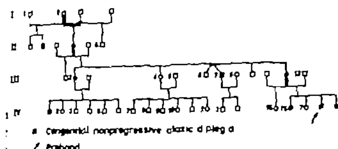



*Fig 1* Pedigree showing congenital nonprogressive ataxic diplegia in three generations

within this group and less than 19 years of age a minimum figure for the proportion of familial forms of congenital ataxias with moderate or severe mental retardation will be 16

Since the number of children per family in Sweden is usually small most cases of autosomal recessive hereditary diseases occur sporadically During the last two decades the number of children per fertile marriage has been between two and three and the percentage of isolated non familial autosomal recessive cases should be approximately two thirds of all cases with that mode of inheritance Thus the proportion of inherited forms of congenital ataxia with mental retardation is even higher or approximately 50 per cent This figure means that if a child is born who proves to be mentally retarded and to have a congenital cerebellar ataxia, w must be aware of the risk that the condition may be genetically determined particularly if the parents are related

*Ataxic diplegia*

Ataxic diplegia was defined according to Ingram (22) meaning a cerebellar ataxia combined with spastic pareses mainly of the l gs

At the physical examination nothing was found that could separate the hereditary ca e from others with a different etiology

We found three families in two families two sibling had an identical form of congenital non progressive ataxic diplegia A 16 year old girl and her 12 year old brother both high grade mental defectives and with healthy parents have been reported previously by Bilh & Hagberg (8) The other two siblings a boy and a girl had in addition to congenital non progressive ataxic diplegia a cellular immunological defect proved for the girl (20) and highly suspected for the boy These siblings showed subnormal intellectual development In neither of these two families was any consanguinity found and the parents were healthy

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and he worked as a farm hand until he retired. Now at 77 years he exhibited slight spasticity of the legs. In addition he showed unsatisfactory balance and dysmetria of the legs, a finding which was difficult to evaluate in an old man but which in this case probably indicated a well compensated congenital ataxia.

A male cousin of the proband (IV 1) had similar slight troubles during his first years of life. He was, however, able to do his military service.

All members of this family affected by ataxic diplegia had an intellectual capacity within the normal range even if the intellectual level was somewhat below the average in some individual patients. In spite of their handicap all had been able to function socially. In the family there were also other clinical aberrations: the proband had a half-sister (IV 15) and a male first cousin (IV 2) who were moderately mentally retarded, the latter furthermore with a cleft palate.

As can be seen from the pedigree the affected persons had affected and normal offspring in about equal proportions. This, together with the fact that the descendants of unafflicted family members were free of the disease, agrees with a dominant mode of inheritance. However, it is impossible to state whether the condition was autosomally or sex-linked inherited. Some of the members were only slightly affected, suggesting a varying degree of expressivity.

Ingram (22) pointed out that ataxic diplegia is a well defined clinical entity, but it is nevertheless difficult to find these cases in the literature because of the variations in classification. Many cases of ataxic diplegia have been hidden in the group of "mixed cerebral palsy" or presumably misinterpreted as simple spastic diplegias. According to Ingram (22) prenatal factors are the cause of ataxic diplegia in about 50 per cent. He reported two families (23) where members in two or more generations had been affected by ataxic diplegia, suggesting a simple dominant mode of inheritance.

In another family Wolfslast (41) reported 5 men with stationary spastic diplegia. They also showed signs of cerebellar involvement, suggesting that these cases were in fact ataxic diplegias. The mode of inheritance in that family was shown to be sex-linked recessive.

A similar clinical picture and a sex-linked inheritance were exhibited by several males in a family reported by Blumel et al. (9). In this family two brothers had essentially spastic paraplegias, but also cerebellar pathway involvement, indicating ataxic diplegia. It was not clear from the report, however, whether the condition was progressive or not. According to our definition of cerebral palsy these case may fall outside the cerebral palsy group.

On the basis of different reports from the literature and our own observations it is quite obvious that genetically determined congenital and nonprogressive ataxic diplegias exist. The usual mode of inheritance is either autosomal recessive or autosomal dominant. The reports of Wolfslast (41) and Blumel et al. (9) also indicate the possibility of a sex-linked recessive variant in individual families.

Our patients with presumptive autosomal recessive inheritance of ataxic diplegia were all moderately mentally retarded. In those with the dominant form the intellectual capacity was within the normal range but in one of Ingram's families (23) the affected members were moderately mentally retarded.

### Spastic diplegia

Spastic diplegia means to us spastic paresis more or less symmetrically distributed and mainly affecting the legs but also to a minor degree the upper extremities.

Our material included a family in which the proband was a 5-year-old boy with a pure spastic diplegia. His parents were not affected but the maternal grandfather was said to have a stationary gait disturbance, exactly like the patient's and present since infancy. Unfortunately, we were not allowed to examine this grandfather, but these cases might be examples of a sex-linked recessive inherited form of spastic diplegia.

In our primary unselected series of cases there was a report concerning two pairs of siblings, all teenagers, with an apparently non-progressive congenital spastic diplegia. However, in both these sibships the eldest of the siblings had shown progressive retinal degeneration during the last few years, a sign possibly indicating a progressive neurologic disorder. We excluded these patients as they did not belong with certainty to the cerebral palsy group.

Several studies of twins with cerebral palsy have been carried out In some studies no pairs of identical twins with the same syndromes have been found (15) while other investiga have revealed identical clinical pictures of spastic paraplegia or diplegia in identical twins (3 4 35 38) In some of these reports it is not clearly stated whether the condition is stationary or not Almost all these patients also had a moderate or severe mental retardation Other authors have discussed heredity in general terms as a causative factor in spastic diplegia and some of them have also reported siblings with spastic diplegia (23 33 43)

Penrose (32) found in a series of mentally retarded per ons 9 pairs of siblings with spas tic diplegia He found consanguinity in 6 of the 65 families with diplegic members In some of the cases the course was shown to be progres sive but there still remained a group which cannot be classified as anything else than cere bral palsy syndromes

Adler (2) found in his study a pair of non identical twins similarly affected by spastic di plegia but with almost normal mental development The parents were related

Book (11) and Hanhart (19) in population isolates reported large family trees of which several members were affected with congenital stationary spastic diplegia associated with e vere mental retardation

Gudmundsson (16) in a study of cerebral palsy in Iceland found consanguinity in 6 4 of the parents of the patients in his series Fur thermore in three families two or more sib lings had spastic diplegia associated with men tal retardation In one of these families the par ents who were related to a very high degree had all their four children severely affected

The literature gives strong evidence for in herited factors as one cause of spastic diplegia especially when combined with mental retar dation In all families reported hitherto the mode of inheritance has probably been auto somal recessive Our family mentioned above suggests the possibility also of sex linked re cessive inheritance of spastic diplegia

*Spastic tetraplegia*

Spastic tetraplegia means to us severe symmet rical or asymmetrical spastic pareses of all four limbs the arms being affected to an equal or greater extent than the legs

In an otherwise healthy sibship we found two sibling 21 and 22 years old with severe spastic tetraplegia associated with moderate to severe mental retardation The parents who were non related and healthy had altogether 11 children The pregnancy and the perinatal history of the two affected sibling were quite normal and the course was non progressive We consider this family to be an example of spastic tetraplegia with a probable autosomal rece sive mode of inheritance

Adler (2) reported a family with 3 siblings similarly affected by non progressive spastic quadriplegia and severe mental retardation The parents were first cousins However they also exhibited atrophy of either papilla a finding that might indicate a progressive dis order

It is quite clear that in the large majority of cases of tetraplegia the cause is perinatal brain damage However in a minority of cases pre natal developmental factors are of principal etiological importance and within this group some cases are obviously genetically deter mined

*Dystonic tetraplegia with true microcephaly*

Dy tonic tetraplegia means to us that all limbs are severely affected the muscle tone is chang ing and the movements are mainly determined by neonatal reflexes Spastic signs are not ob ligate but sometimes present

In our material there were two siblings a boy aged 7 years and his sister aged 6 years with identical pictures of typical microcephaly dystonic spastic tetraplegia and severe mental retardation The parents were unrelated and healthy and had no other children. The peri natal period of both siblings was entirely nor mal The siblings had both been followed up continuously and no progression was seen Their appearance was highly characteristic of

microcephaly The head circumference in the boy was 49 5 cm and in the girl 48 5 cm at the ages given above

The descriptive entity microcephaly comprises several etiologically different varieties Genetically determined forms of microcephaly are well known (10 12, 26) They usually have an autosomal recessive mode of inheritance These inherited forms of microcephaly are not usually combined with cerebral palsy (12 26) although Book (12) found a certain proportion with what he called generalized spasticity

Davies & Kirman (13) however found a high proportion of cerebral palsy ( spasticity ) in a series of microcephalics the microcephalic siblings often being similarly affected

Adler (2) reported two families with siblings identically affected with microcephaly associated with spastic quadriplegia and severe mental retardation

Thus, even a group comprising genetically determined microcephaly combined with cerebral palsy certainly includes several genetic and clinical entities Our family reported above seems to represent one of them, exhibiting a very characteristic clinical picture

The mode of inheritance seems to be autosomal recessive for this special type as well as in the families with a similar type of microcephaly and tetraplegia reported by Adler (2)

### *The proportion of simply inherited cases of cerebral palsy in relation to all cases*

The proportion of simply inherited cases of cerebral palsy in relation to the total group of cerebral palsy in Sweden can only be calculated approximately from this investigation as reliable frequency figures of cerebral palsy for persons 19 years or older are still lacking in Sweden In the whole country the number of persons with cerebral palsy below 19 years of age was calculated to be 3150 in 1960 (40) In our material there were 15 familial cases with presumably autosomally recessively inherited forms of cerebral palsy below 19 years of age, which gives a minimum figure of 0 5 per cent This figure represents only one third [of] the true proportion for the same reasons a[s] given concerning the calculation of inherited congenital ataxia (p 335) Thus it is estimated that approximately 1 5 per cent of all cases of cerebral palsy in Sweden are autosomally recessively inherited The sex linked recessive and dominant forms seem to be much more rare

However Ingram s experiences (?3) and preliminary results of investigations made by Glenting Copenhagen (14) suggest that more complicated and multifactorial modes of inheritance may numerically be more important especially for the group with spastic diplegia Thus heredity constitutes one obvious etiological factor in cerebral palsy which even if un[common] cannot be ignored

## SUMMARY

Familial cases of cerebral palsy were traced all over Sweden Fortythree families were collected, in 30 of which the patients were siblings The families were divided into three groups (1) 16 families with cases of identical syndromes and a history of normal pregnancy delivery and perinatal period (2) 3 families with cases of identical syndromes but an abnormal perinatal period (3) 24 families with non identical syndromes

Within the first group, which is of main genetic importance 10 families were found with 2–3 siblings affected with congenital non progressive ataxia and mental retardation the mode of inheritance with all probability being autosomal recessive Three families showed ataxic diplegia two of them only in siblings the third with affected members of both sexes represented in three generations Surprisingly enough pure spastic diplegia was only revealed in one family viz a grandfather and his grandson Spastic tetraplegia was found in two mentally retarded siblings in an otherwise healthy sibship of 11 members True microcephaly combined with a dystonic tetraplegic cerebral palsy was seen in one family and was

ought to have an autosomal recessive inheritance as in similar cases reported in the literature.

Chromosome studies and laboratory screening tests revealed no abnormalities indicating particular aetiological mechanisms.

## ACKNOWLEDGEMENTS

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# MAPLE SYRUP URINE DISEASE

## *Four Years Experience with Dietary Treatment of a Case*

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Maple Syrup Urine Disease (M S U D) is an inborn error of metabolism first described by Menkes Hurst & Craig in 1954 It is characterized clinically by the onset of signs of intracranial disturbance during the first weeks of life and biochemically by very high systemic and urinary concentrations of leucine isoleucine and valine and of their corresponding 2 oxoacids associated with a characteristic odour These symptoms progress and death may supervene at any time Although apparently a rarity the clinical importance of M S U D lies in the facts that death may be indefinitely postponed by suitable dietary treatment and that mental retardation need not be an inevitable sequela if dietary therapy is instituted at a sufficiently early stage

The critical nature of the early period is emphasized by our experience in treating a girl with M S U D from the age of six weeks She is now four and a half years old there is a considerable degree of mental retardation presumably related to the delay in instituting dietary therapy

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## CASE REPORT

The parents are healthy and unrelated Their first born a male had a large meningomyelocele and died aged five weeks The second child is a healthy six year-old girl

*First week*

The affected infant was born at home on 26th May *1964* wei hing 3150 g at 39 weeks maturity Pregnancy and delivery were normal She appeared wel until the 5th day when she was slow to feed on th 6th day she was too lethargic to suck at the breas She was admitted to hospital in a flaccid pale an very drowsy condition weighing 2950 g on the 7t day Her semicomatose state was interrupted occa sionally with restlessness associated with a high pit hed cry The head circumference was 34 cm th fontanelle convex and rather tense and the suture easily palpable Respiration was 40 per minut breathing being irregular and stertorous The chi was not cyanosed and the heart and chest were no *mal* The liver and spleen were both palpated 1 c below the costal margins The laboratory investig tions performed at this time showed only an abno mal haematological picture which did not aid dia nosis and a mild urinary tract infection for whi intramuscular streptomycin and penicillin were giv

During the *7th and 8th days* of life the baby h four convulsions in which she champed and clench her jaws adopted bizarre postures waving her lim but not actually twitching, and became a grey colo The convulsions were controlled with chloral (30 m 3 hourly) but the general condition of the baby not *improve* The urinary tract infection persisted a on the 10th day of life the antibiotics were chan to nitrofurantoin (60 mg 6-hourly) by the oral route

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Key words Cerebral palsy familial form cerebral palsy ataxia ataxic diplegia

Table 1. *Plasma aminoacid and 2-oxoacid concentrations*

Plasma aminoacid concentrations are given in mg/100 ml and 2-oxoacid concentrations in mEq/l

| | Tau | Glu | Pro | Gly | Ala | Val | Met | Cys | allo Ileu | Ileu | Leu | Tyr | Phe | Orn | Lys | His | Arg | 2-oxo acid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *a. From diagnosis to establishment of control* | | | | | | | | | | | | | | | | | | |
| Day of life | | | | | | | | | | | | | | | | | | |
| 43 | | | | | | | | | | | | | | | | | | 1 84 |
| 44 | | 17 | 18 | 07 | 03 | 102 | 01 | 05 | 23 | 111 | 73 | 14 | 21 | 07 | 12 | 06 | 11 | |
| 50 | | 26 | 12 | 64 | 09 | 08 | 23 | 09 | 26 | 18 | 909 | 30 | 26 | 04 | 15 | 07 | 04 | |
| 4 | | 28 | 13 | 28 | 06 | 11 | 13 | 11 | 23 | 14 | | 15 | 18 | 03 | 06 | 07 | 03 | 0 63 |
| 56 | | 7 | 11 | 53 | 14 | 09 | 11 | 09 | 15 | 01 | 235 | 19 | 21 | 08 | 12 | 09 | 03 | 0 55 |
| 63 | 09 | 3 | 18 | 58 | 13 | 06 | 13 | | 58 | 98 | 94 | 19 | 15 | 05 | 16 | 08 | 06 | 0 18 |
| 67 | 83 | 29 | 28 | 91 | 4 | 07 | 19 | | 69 | 112 | 61 | 26 | 23 | 07 | 30 | 11 | 30 | |
| 7 | 11 | | | 10 | 48 | 06 | | | 69 | 32 | 24 | 25 | 20 | 08 | 42 | 14 | 17 | |
| 1 0 | 06 | 26 | 34 | 61 | 31 | 84 | 14 | 13 | 29 | 19 | 03 | 21 | 07 | 13 | 32 | 12 | 15 | 0 12 |
| *b. During the course of an acute infection* | | | | | | | | | | | | | | | | | | |
| Date | | | | | | | | | | | | | | | | | | |
| 11 5 | 15 | | 19 | 43 | 16 | 30 | 12 | 10 | 13 | 5 | 81 | 12 | 11 | 09 | 19 | 11 | 67 | |
| 10 6 | 14 | | 20 | 66 | 20 | 43 | | | 17 | 25 | 155 | 17 | 13 | 13 | | | | |
| 14 6 | 17 | | 13 | 06 | 07 | 114 | | 17 | 17 | 73 | 215 | 16 | 19 | 27 | 31 | 11 | 14 | 6 97 |
| 16 6 | | | 1 | 18 | 06 | 140 | | | 26 | 86 | 960 | 07 | 11 | 13 | 19 | 09 | 08 | 0 84 |
| 1 6 | | | 13 | 84 | 14 | 11 | | | 08 | 10 | 106 | 14 | 08 | 08 | 23 | 09 | | |
| 7 6 | 13 | 10 | 14 | 28 | 19 | 08 | | | 06 | 06 | 21 | 03 | 04 | 06 | 11 | 09 | 04 | |

baby had gained no weight and the stools were loose. There was also an erythema around the buttocks which it was thought had been caused by the frequent use of urine collecting bags. The baby was [illegible] to take feeds by bottle satisfactorily, head control was improving and the fontanelle less tense. The anaemia and associated reticulocytosis persisted and a bone marrow biopsy was performed which showed some degree of erythropoietic hypoplasia. The urine was no longer infected. A diet of Mixture [illegible] continued supplemented by other aminoacids which appeared in low concentrations on the plasma aminoacid chromatogram.

*[illegible] second stage of treatment*

Twenty five days after the institution of dietary therapy (day 77) the concentration of leucine had been [illegible] to within normal limits and the concentration of isoleucine alone and other aminoacids were [illegible] satisfactory. At this time the composition of the commercial Maple Syrup Mixture No. 1 was still unsuitable and so the previous diet was continued with the addition of 100 mg each of leucine and isoleucine and 100 mg of valine daily. Two weeks after the introduction of these supplements (day 90) the plasma leucine concentration had risen to 12 mg/100 ml and leucine was omitted from the diet for the next ten days during which the concentration fell to [illegible] more to normal.

*[illegible] overt vitamin deficiency*

Six weeks after dietary treatment had been started (day 88) the baby's condition remained a matter for concern. There had been no weight gain and despite the transfusion given at the onset of dietary treatment the anaemia unaccompanied by reticulocytosis progressed. Additionally there was exacerbation of the napkin rash and a sudden development of angular stomatitis. On checking the preparation of the diet at the pharmacy it was discovered that since the third day of dietary treatment the vitamin mixture described by Westall (70) containing B [illegible] biotin, folic acid, choline chloride, 4-aminobenzoic acid, inositol and pantothenic acid had been omitted. At this time (95th day) the serum B [illegible] concentration was 576 [illegible] per ml (normal—160–925 [illegible]/ml) and serum folate was 2 mµg 100 ml (normal—6 0–21 0 mµg/100 ml).

The vitamin supplements were re-introduced on the 95th day and in addition 3 g of fresh baker's yeast were incorporated into the daily diet. Three days later the haemoglobin concentration had fallen to 4.5 g/100 ml [illegible] with only [illegible] reticulocytes and it was thought unwise to wait longer for haematological response to vitamin therapy alone. A transfusion of 82 ml of packed red cells was given on the 99th day. Three days later the child was much improved. She was [illegible] more alert and although there had been no increase in weight the buttocks were less red and the angular stomatitis was in process of clearing.

*F [illegible] third stage—introduction of new materials*

On the 99th day the plasma leucine concentration had fallen to 1 5 mg/100 ml and 35 ml of fresh cow's milk were added to the daily diet. A week later the concentration was 0 3 mg/100 ml and it was considered that Maple Syrup Mixture No. 1 could be introduced and the double cream was replaced by arachis oil. Cornflour starch was introduced and

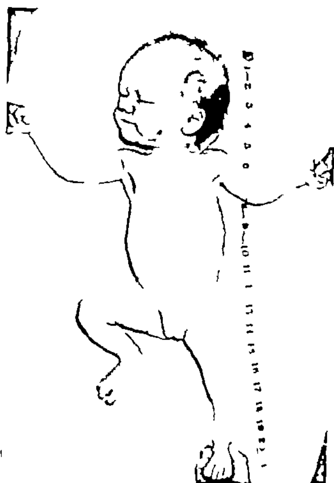


Fig 1a

*Second to fourth weeks*

Throughout this period the baby was very drowsy feeding by gavage was required and temperature was unstable She gave a high pitched cry only when disturbed The Moro reflex was absent the grasp reflex weak and the tendon reflex depressed The pupils were equal and reacted normally to light Breathing was irregular and occasionally stridor was noted in association with excessive buccal secretions The fontanelle was tense and the head circumference increased by 1 cm during the second week On the 14th day bilateral subdural taps revealed no haematoma At 21 days a right ventricular puncture revealed normal fluid pressure

*Fifth to seventh weeks*

At 28 days of age the baby was transferred to a medical ward for children in another hospital and the description recorded on admission was essentially a summary of the findings recorded above She lay in a state of extreme drowsiness (Fig 1 *a*) and showed marked hyporeflexia

A few days after transfer the ward sister reported that the baby had an unpleasant sweet yeasty smell and this eventually prompted a request for chromatography of the urinary aminoacids The paper chromatogram showed very dense spots of leucine isoleucine and valine and an unidentified spot in the position of methionine this pattern typical of an untreated case of MSUD A strongly positive reaction for keto compounds in the urine was obtained and urine specimens at this time (ca 35 days) smelled strongly of maple syrup A high urinary white cell count (e g 620 per mm³) and bacteriuria persisted despite treatment with sulphadimidine oxytetracycline and nitrofurantoin Control of the infection by intramuscular streptomycin coincided with the establishment of dietary control Sulphonamides were given prophylactically up to the 70th day of life and there has been no recurrence of the urinary tract infection

When the condition had been diagnosed estimates were made of the plasma aminoacid and oxoacid concentrations and urinary aminoacid and oxoacid excretions The most striking abnormality was a fasting plasma leucine concentration of 73 mg per 100 ml The isoleucine and valine concentrations were less markedly raised (Table 1 *a*)

*Seventh week first stage of treatment*

On the 47th day treatment aimed at rapidly reducing the grossly raised concentrations of the branched chain aminoacids leucine isoleucine and valine was instituted The child received virtually no leucine or valine for six days but was in receipt of the other essential and non-essential aminoacids in the commercial Maple Syrup Mixture No 2 (Scientific Hospital Supplies Liverpool Ltd Table 3) During the time the concentrations of isoleucine and valine in the plasma fell to within normal limits and sufficient was added to the diet as pure L aminoacids (about 350 and 160 mg/day respectively) for maintenance of normal plasma concentrations and for protein synthesis

During this stage of treatment the exact composition of the commercial Maple Syrup Mixture No 1 which contains appreciable quantities of the branched chain aminoacids was not available and it was considered unwise to introduce it to the diet Other dietary requirements were satisfied with double cream sucrose and mineral and vitamin mixtures

Three days after the diet was started the baby showed increasing interest in her environment made spontaneous sucking movements and took her first feed by bottle for many weeks Nonetheless she was still drowsy the fontanelle remained full and the urinary infection persisted She was anaemic (haemoglobin 6 4 g/100 ml reticulocytes 1 8 ) and had moderate hepatosplenomegaly On the fifth day of treatment (day 51) pallor was pronounced (haemoglobin 5 0 g/100 ml reticulocytes 10 8 ) and a transfusion of concentrated red cells (74 ml) was given

Nine days after the start of the dietary regime (day 56) the baby was taking all feeds by bottle and was much more restless often throwing the head back there were inco ordinate eye movements and flickering of the eyelids The fontanelle remained full and the liver and spleen easily palpable Twelve days from the start of the dietary treatment (day 59) the

Table 1 *Plasma aminoacid and 2-oxoacid concentrations*

Plasma aminoacid concentrations are given in mg/100 ml and 2-oxoacid concentrations in mEq l

| | Tau | Gln | Pro | Gly | Ala | Val | Met | Cys | allo Ileu | Ileu | Leu | Tyr | Phe | Orn | Lys | His | Arg | 2-oxo acid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *From diagnosis to establishment of control* | | | | | | | | | | | | | | | | | | |
| Day of life | | | | | | | | | | | | | | | | | | |
| 43 | | | | | | | | | | | | | | | | | | 1 84 |
| 46 | | 17 | 18 | 07 | 03 | 102 | 01 | 05 | 23 | 111 | 73 | 14 | 21 | 07 | 12 | 06 | 11 | |
| 50 | | 76 | 17 | 64 | 09 | 08 | 23 | 09 | 26 | 18 | 509 | 30 | 26 | 04 | 15 | 07 | 04 | |
| 52 | | 28 | 13 | 28 | 06 | 11 | 13 | 11 | 23 | 14 | | 15 | 18 | 03 | 06 | 07 | 03 | 0 63 |
| 56 | | 27 | 11 | 53 | 14 | 09 | 11 | 09 | 15 | 01 | 235 | 19 | 71 | 08 | 12 | 09 | 03 | 0 55 |
| 63 | 09 | 37 | 18 | 58 | 13 | 06 | 13 | | 38 | 98 | 94 | 19 | 15 | 05 | 16 | 08 | 06 | 0 18 |
| 61 | 0 | 29 | 28 | 91 | 24 | 07 | 19 | | 69 | 112 | 61 | 26 | 23 | 07 | 30 | 11 | 30 | |
| 7 | 11 | | | 10 | 48 | 06 | | | 69 | 32 | 24 | 25 | 20 | 08 | 42 | 14 | 17 | |
| 170 | 06 | 26 | 34 | 61 | 31 | 84 | 14 | 13 | 29 | 19 | 03 | 21 | 07 | 13 | 32 | 12 | 15 | 01 |
| *b During the course of an acute infection* | | | | | | | | | | | | | | | | | | |
| Date | | | | | | | | | | | | | | | | | | |
| 11 5 | 15 | | 19 | 43 | 16 | 0 | 12 | 10 | 13 | 25 | 81 | 12 | 11 | 09 | 19 | 11 | 07 | |
| 10 6 | 14 | | 20 | 66 | 20 | 43 | | | 17 | 25 | 155 | 17 | 13 | 13 | | | | |
| 14 6 | 17 | | 13 | 06 | 07 | 114 | | 17 | 17 | 73 | 215 | 16 | 19 | 27 | 31 | 11 | 14 | 0 97 |
| 16 6 | | | 12 | 10 | 06 | 140 | | | 26 | 86 | 360 | 07 | 11 | 13 | 19 | 09 | 08 | 0 84 |
| 1 6 | | | 13 | 84 | 14 | 11 | | | 08 | 10 | 106 | 14 | 08 | 08 | 23 | 09 | | |
| 7 6 | 13 | 10 | 14 | 8 | 19 | 06 | | | 06 | 06 | 21 | 03 | 04 | 06 | 11 | 09 | 04 | |

baby had gained no weight and the stools were loose. There was also an erythema around the buttocks which it was thought had been caused by the frequent use of urine collecting bags. The baby was continuing to take feeds by bottle satisfactorily, head control was improving and the fontanelle less tense. The anaemia and associated reticulocytosis persisted and a bone marrow biopsy was performed which showed some degree of erythropoetic hypoplasia. The urine was no longer infected. A diet of Mixture 2 was continued supplemented by other aminoacids which appeared in low concentrations on the plasma aminoacid chromatograms.

*Ten weeks second stage of treatment*

Twenty five days after the institution of dietary therapy (day 77) the concentration of leucine had been reduced to within normal limits and the concentrations of isoleucine, valine and other aminoacids were also satisfactory. At this time the composition of the commercial Maple Syrup Mixture No 1 was still unavailable and so the previous diet was continued with the addition of 100 mg each of leucine and isoleucine and 100 mg of valine daily. Two weeks after the introduction of these supplements (day 86) the plasma leucine concentration had risen to 12 mg/100 ml and leucine was omitted from the diet for the next ten days during which the concentration fell once more to normal.

*Thirteenth week onset of vitamin deficiency*

5 weeks after dietary treatment had been started (day 81) the baby's condition remained a matter for concern. There had been no weight gain and despite the transfusion given at the onset of dietary treatment the anaemia unaccompanied by reticulocytosis, progressed. Additionally there was exacerbation of the napkin rash and a sudden development of angular stomatitis. On checking the preparation of the diet at the pharmacy it was discovered that since the third day of dietary treatment the vitamin mixture described by Westall (70) containing B₁₂, biotin, folic acid, choline chloride, 4 aminobenzoic acid, inositol and pantothenic acid had been omitted. At this time (95th day) the serum $B_{12}$ concentration was 576 µµg per ml (normal—160-925 µµg/ml) and serum folate was 2 mµg/100 ml (normal—6 0–21 0 mµg/100 ml).

The vitamin supplements were re introduced on the 95th day and in addition 5 g of fresh baker's yeast were incorporated into the daily diet. Three days later the haemoglobin concentration had fallen to 4 3 g/100 ml (32 %) with only 0 8 reticulocytes and it was thought unwise to wait longer for haematological response to vitamin therapy alone. A transfusion of 82 ml of packed red cells was given on the 99th day. Three days later the child was much improved. She was generally more alert and although there had been no increase in weight the buttocks were less red and the angular stomatitis was in process of clearing.

*Fifteenth week third stage—introduction of new materials*

On the 99th day the plasma leucine concentration had fallen to 1 5 mg/100 ml and 35 ml of fresh cow's milk were added to the daily diet. A week later the concentration was 0 3 mg/100 ml and it was considered that Maple Syrup Mixture No 1 could be introduced and the double cream was replaced by arachis oil, cornflour starch was introduced and



Fig 1b

1 g of arginine was added to the daily diet. From this time (112th day) the baby began to gain weight and became hungry and more alert and active (Fig 1 b). The calorie content of the diet was increased and more Maple Syrup Mixture No 1 was given to provide adequate leucine isoleucine and valine for protein synthesis. As the infant's appetite increased (122nd day) tinned foods of low protein content were introduced and later varying amounts of whole egg. For some time the plasma leucine concentration was effectively controlled by adding or subtracting quantities of egg from the daily intake.

Further blood transfusions were not required, the haemoglobin concentration being well maintained despite frequent blood sampling for plasma aminoacid estimations.

*Electroencephalograms*

A series of eight electro-encephalograms were recorded from our patient between the ages of five weeks and five months. The electrographic pattern fluctuated widely in the earlier records and showed an excessive amount of delta activity frequently of asymmetrical distribution over the hemispheres; the asymmetry was sufficiently pronounced in the first record to raise the question of a subdural collection of fluid in the left parietal area. Following dietary therapy the pattern returned gradually to normal and the rhythms in later recordings appeared normal for the age of the patient. It would seem that the electroencephalogram shows no distinctive features of value in diagnosing M S U D.

*Long term management*

From the age of six months to four years the patient was largely managed at home by her parents. She attended as an out patient every two or four weeks for estimation of plasma aminoacid concentrations, the mother collecting further supplies of the diet at the same time. Frequent alterations in the diet have been unnecessary during periods of good health. The child suffered a number of respiratory infections and otitis media which necessitated re-admission to hospital to facilitate the treatment and adjustments in the diet necessary to control the plasma pH and reduce the elevated concentrations of branched chain aminoacids. This problem is discussed later. There has been no recurrence of the urinary tract infection which was such an intractable feature during the period before dietary treatment. Except during periods of illness the child's weight continued to be satisfactory.

The patient is now four and a half years old. She weighs 11.5 kg, height 94 cm. She is a cheerful, well nourished but slender infant; there is a moderate degree of microcephaly (head circumference 47 cm) and some degree of general spasticity of the limbs. Although the degree of mental retardation is difficult to assess, it is probably considerable; her behaviour is that of an eighteen month-old infant.

## INVESTIGATIONS

Laboratory investigations were carried out by standard methods. Plasma aminoacid concentrations were estimated on 4 hr fasting venous samples deproteinised with sulphosalicylic acid using the standard 22 hr chromatogram procedure on a Technicon Amino Acid Analyser. Plasma and urine 2 oxoacids were estimated by the method of Friedman & Haugen (22) using pyruvic acid as standard. Urinary 2 oxoacids were identified by chromatography following conversion to the parent aminoacids by electrolytic reduction of the 2, 4 dinitrophenyl hydrazones.

## DISCUSSION

*Presentation and diagnosis*

In this disease deterioration is rapid and follows a fairly well established pattern. Initial lethargy and refusal to feed often with associated vomiting are quickly followed by signs of progressive and severe intracranial disturbances in the present case taking the form of stertorous breathing, intermittent opisthotonus, hypotonia and hyporeflexia with absent sucking and Moro reflexes.

The diagnosis may be suggested by the striking symptomatology and the unusual timing of onset of the intracranial disturbances but it is the characteristic odour of the urine which is most suggestive if appreciated. Although not initially recognised in our case the odour was readily appreciated during subsequent lapses in control and during the course of acute infections. It has also been observed that the wax from the external auditory canals has the very characteristic odour akin to burnt sugar or maple syrup even in the absence of the characteristic urinary odour during periods of good dietary control.

As the prospects for normal mental development are dependent upon early diagnosis and institution of dietary therapy a primary metabolic disturbance of this type should be considered in the differential diagnosis of all babies in whom unsatisfactory progress towards the end of the first week of life is unexplained and laboratory investigations to establish such a diagnosis should be undertaken at the same time that the more common causes of intracranial disturbances are being eliminated.

The laboratory investigations to confirm the diagnosis of M.S.U.D. involve quantitative or semiquantitative estimations of the concentrations of the branched chain aminoacid leucine, isoleucine and valine in urine or plasma or both together with the identification of the 2-oxoacids derived from these aminoacids by transamination in the same material (5, 20, 59). Where facilities for such investigations are not immediately available a strongly positive reaction of the patients urine with 2,4-dinitrophenylhydrazine linked to a suggestive clinical history or even the suggestive history alone is sufficient indication for the institution of dietary therapy involving total protein restriction.

During the past two years the plasma amino acid concentrations of 63 children other than the present patient of less that two years of age have been accumulated, 67 analyses in all. These children have been investigated because of failure to thrive or fits or because sibs have had inherited aminoacidopathies. The blood specimens have been taken at any time between one and a half and five hours after the last previous meal. The values obtained have been assessed as log normal distributions and the figures below are the means and lower and upper 95% confidence limits. The author feel that these figures are better criteria for a decision concerning the normality or abnormality of a result obtained from a sick infant than figure derived from carefully controlled assessment of normal children.

| | Mean | 95% range |
|---|---|---|
| Valine mg/100 ml | 2.56 | 1.05–6.18 |
| Isoleucine mg/100 ml | 0.87 | 0.35–2.19 |
| Leucine mg/100 ml | 1.58 | 0.64–3.83 |

*Prognosis*

Fifty five infants suffering from this disease have now been formally reported and many others recorded as hearsay. It is frequently noted that from families bearing a patient with M.S.U.D. histories of other children dying in infancy with similar symptoms have been elicited but these are not included in this total. It is probable that, because of the acute nature of the disease many cases are not recognised and it is unlikely that the reported cases even approximate the true incidence of the disease. However the reported cases have been widely distributed both geographically and among the different racial groups. Additionally there are reports of seven instances and mention of others of a variant of the disease which is not neccessarily lethal *per se* if untreated and

does not inevitably produce mental retardation and in which the chemical symptoms are manifested spasmodically (7 17 34 39 46 47)

Thirty of the reported cases received no special dietary treatment (1, 2 3 5 10–16 21 29 32 33 35 36 37 39 40 44 48 51 53, 55 57 58 59 63 67 69 73 74 75) For some dietary treatment was not considered the materials for feeding restricted amounts of the branched chain aminoacids to others were not available on many occasions the diagnosis was made only after the death of the patient

All except two of the patients who received no dietary treatment died ages at death ranged from three days to 20 months The exceptions (13 15) were alive at 15 month and 13 years of age and are thus classifiable tautologically as examples of the late manifesting variant However the patients were never well and were much retarded unlike other cases of the variant Not one of the untreated patients made any progress and in those instances where necropsy was performed characteristic changes in the brain tissue were found

In contrast eight of the 25 cases given dietary treatment were alive at the time of their last being reported

Four of these survivors were reported to be mentally and developmentally normal at the age of three months three three and a half and seven years (21 27 31 38 61 63 70 71) for these patients rigid dietary control was begun before the 10 th day of life In a further case (28 60 61 62 63) treatment was started during the third week of life with restriction of methionine as well as branched chain amino acids despite improvement in the general condition, the patient has remained retarded though still alive at seven years of age Treatment from six weeks in the present case and from five weeks and nine months in others (63 15) had not reversed the retardation at the ages of four years eight months and three years respectively

Of those who had received dietary treatment and had died only one (36) treated from ten days appeared to develop normally until death at four and a half months Two sibs treated from six days and one month (24 54) made no progress and died at six months a further case treated from two months, died at two years much retarded (55 56 57) even though the diets of all three appear to have been well controlled Two further cases (4 26) treated from six weeks and one year died at seven months and nearly three years of age respectively both appeared to have been well controlled and had improved in general health though there was still marked development of retardation Four other case though treated did not survive the initial onset (2 15 21 29) The remainder (5 12 15 21 36 37 39 40 51 52 63 68 69 74) were not subjected to the rigid control of intake of branched chain aminoacids that seems necessary and were treated from two months or more of age Each deteriorated and most succumbed during ostensibly mild infections

In general it would appear that for those infants who survive the first onset of symptoms there is a critical period between two and four weeks of age after which persisting metabolic disturbances causes permanent damage to the nervous system

*Some consequences of the biochemical lesion*

The large systemic concentrations of 2-oxo-acids found in untreated cases of M S U D arising from the deficiency of the 2 oxoacid dehydrogenases concerned (9 12 23 25) together with the large concentrations of 2 hydroxy acids represent a large pool of non metabolisable acid material which causes a gross systemic metabolic acidosis The acid base balance of our patient was not investigated at the initial presentation, but severe acidosis has been a constant feature of subsequent infections For our patient the estimated concentration of 2 oxo and 2 hydroxy acids was of the same order as the base deficit Thus on 14th June 1968 (Table 1*b*) the plasma 2 oxo acid concentration was 0 97 mEq/l this implies a 2 hydroxy acid concentration of ap-

Table 2 *Renal clearances of branched chain amino acids*

Clearances uncorrected for surface area Endogenous creatinine clearance 7 ml/min

| Aminoacid | Plasma concentration mg/100 ml | Clearance ml/min |
|---|---|---|
| Val | 5 0 | 0 01 |
| | 14 3 | 0 05 |
| allo-Ilu | 1 3 | 0 04 |
| | 2 6 | 0 05 |
| Ilu | 2 6 | 0 02 |
| | 8 6 | 0 05 |
| Leu | 8 1 | 0 01 |
| | 36 0 | 0 08 |

proximately 10 mEq/l At this time the patient had a blood pH of 7 23 $P_{co_2}$ 30 mm Hg and standard bicarbonate 13 4 mEq/l giving a base deficit of 14 2 mEq/l (Calculated as suming the pyruvate lactate ratio which is approximately 1–10 applies to other oxo and hydroxy acid systems )

The acidosis and the presence of large con centrations of the 2 hydroxy and 2 oxo acids can disturb nerve cell function even if this were previously normal as demonstrated by the cases of the Late Manifesting Variant These patients developed the biochemical and neurological features of M S U D during periods of infection or after trauma This im plies that, unlike phenylketonuria dietary con trol can never be completely relaxed and this has to some extent been confirmed (71 72) Another observation of practical importance is the experience of Morris *et al* (46) with a case of the Late Manifesting Variant which suggests that even minor surgery may be ex tremely hazardous

In addition to acute toxicity the branched chain amino acids or their derivatives may re tard the maturation of brain tissue and per manently disturb intellectual function (41 42 43 44 45 48) This may be by inhibiting mye lin synthesis or by producing an abnormal myelin under the influence of 2-oxo and 2 hydroxy acids

Table 3 *Composition*[1] *of the Maple Syrup Mixtures*

Concentrations in mg/g dry weight

| Aminoacid | I | II |
|---|---|---|
| Asp | 56 | — |
| Thr | 20 | 21 |
| Ser | 32 | — |
| Glu | 93 | 353 |
| Pro | 120 | — |
| Gly | 181 | 353 |
| Ala | 65 | — |
| Val | 27 | — |
| Met | 9 | 41(dl) |
| Cys | 5 | 18 |
| Ilu | 18 | — |
| Leu | 34 | — |
| Tyr | 8 | 62 |
| Phe | 22 | 41 |
| Trp | 17 | 21 |
| Lys | 33 | 59 |
| His | 9 | 32 |
| Arg | 66 | — |

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*Renal clearance and allo isoleucine*

The renal clearances of the branched chain aminoacids have been measured in our patient during periods when the plasma levels were both normal and raised at the age of 20–22 months and are essentially similar to those col lected by Soupart (65) for normal subjects The clearance of leucine against a plasma concen tration of over 30 mg/100 ml is eight times that at 8 mg/100 ml (Table 3) but still only one hundredth of the creatinine clearance and this absence of a threshold at reasonable plasma concentrations must partly account for some of the very high concentrations of the branched chain aminoacids recorded at the time of diagnosis These results confirm the im pression derived from two dimensional paper chromatograms that there is no disturbance of renal reabsorptive fuction in M S U D The re nal clearances of L allo-isoleucine (50) at comparable plasma concentrations are about twice as great as the clearances of L isoleucine The observation that a plasma concentration of allo-isoleucine comparable to that of isoleu cine is maintained by patients with M S U D indicates that the transaminase system for the

does not inevitably produce mental retardation and in which the chemical symptoms are manifested spasmodically (7 17 34 39 46 47)

Thirty of the reported cases received no special dietary treatment (1 2 3 5 10–16 21 29 32 33 35 36 37 39 40 44 48 51 53 55 57 58 59 63 67 69 73 74 75) For some dietary treatment was not considered the materials for feeding restricted amounts of the branched chain aminoacids to others were not available on many occasions the diagnosis was made only after the death of the patient

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| Val | 77 | — |
| Met | 9 | 41(dl) |
| Cys | 5 | 18 |
| Ileu | 18 | — |
| Leu | 34 | — |
| Tyr | 8 | 62 |
| Phe | 2_ | 41 |
| Trp | 17 | 21 |
| Lys | 33 | 59 |
| His | 9 | 32 |
| Arg | 66 | — |

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*Renal clearance and allo-isoleucine*

The renal clearances of the branched chain aminoacids have been measured in our patient during periods when the plasma levels were both normal and raised at the age of 20–22 months and are essentially similar to those collected by Soupart (65) for normal subjects The clearance of leucine against a plasma concentration of over 30 mg/100 ml is eight times that at 8 mg/100 ml (Table 3) but still only one hundredth of the creatinine clearance and this absence of a "threshold" at reasonable plasma concentrations must partly account for some of the very high concentrations of the branched chain aminoacids recorded at the time of diagnosis These results confirm the impression derived from two dimensional paper chromatograms that there is no disturbance of renal reabsorptive fuction in M S U D The renal clearances of L allo-isoleucine (50) at comparable plasma concentrations are about twice as great as the clearances of L isoleucine The observation that a plasma concentration of allo-isoleucine comparable to that of isoleucine is maintained by patients with M S U D indicates that the transaminase system for the

does not inevitably produce mental retardation and in which the chemical symptoms are manifested spasmodically (7 17 34 39 46 47)

Thirty of the reported cases received no special dietary treatment (1 2 3 5 10–16 21 29 32 33 35 36 37 39 40 44 48 51 53 55 57 58 59 63 67 69 73 74 75) For some dietary treatment was not considered the materials for feeding restricted amounts of the branched chain aminoacids to others were not available on many occasions the diagnosis was made only after the death of the patient

All except two of the patients who received no dietary treatment died ages at death ranged from three days to 20 months The exceptions (13 15) were alive at 15 months and 13 years of age and are thus classifiable tautologically as examples of the late manifesting variant However the patients were never well and were much retarded unlike other cases of the variant Not one of the untreated patients made any progress and in those instances where necropsy was performed characteristic changes in the brain tissue were found

In contrast eight of the 25 cases given dietary treatment were alive at the time of their last being reported

Four of these survivors were reported to be mentally and developmentally normal at the age of three months three three and a half and seven years (21 27 31 38 61 63 70 71) for these patients rigid dietary control was begun before the 10 th day of life In a further case (28 60 61 62 63) treatment was started during the third week of life with restriction of methionine as well as branched chain amino acids despite improvement in the general condition the patient has remained retarded though still alive at seven years of age Treatment from six weeks in the present case and from five weeks and nine months in others (63 15) had not reversed the retardation at the ages of four years eight months and three years respectively

Of those who had received dietary treatment and had died only one (36) treated from ten days, appeared to develop normally until death at four and a half months Two sibs treated from six days and one month (24 54) made no progress and died at six months a further case treated from two months, died at two years much retarded (55 56 57) even though the diets of all three appear to have been well controlled Two further cases (4 26) treated from six weeks and one year died at seven months and nearly three years of age respectively both appeared to have been well controlled and had improved in general health though there was still marked development of retardation Four other cases though treated did not survive the initial onset (2, 15 21 79) The remainder (5 12 15 21 36 37 39 40 51 52 63 68 69 74) were not subjected to the rigid control of intake of branched chain aminoacid that seems necessary and were treated from two months or more of age Each deteriorated and most succumbed during ostensibly mild infections

In general it would appear that for those infants who survive the first onset of symptoms there is a critical period between two and four weeks of age after which persisting metabolic disturbances causes permanent damage to the nervous system

*Some consequences of the biochemical lesion*

The large systemic concentrations of 2 oxo acids found in untreated cases of MSUD arising from the deficiency of the 2 oxoacid dehydrogenase concerned (9 12 23 25) together with the large concentrations of 2 hydroxy acids represent a large pool of nonmetabolisable acid material which causes gross systemic metabolic acidosis The acid-base balance of our patient was not investigated at the initial presentation but severe acidosis has been a constant feature of subsequent infections For our patient the estimated concentration of 2 oxo and 2 hydroxy acids was of the same order as the base deficit Thus on 14th June 1968 (Table 1*b*) the plasma 2 oxo acid concentration was 0 97 mEq/l this implies a 2 hydroxy acid concentration of ap-

tern is in accord with the experiences of Westall (71) and others The reason for this sharp decline is readily appreciated when the individual metabolic requirements are considered

Our second point concerns infections A significant number of the reported cases of M S U D have succumbed during the course of relatively minor infections and any infection must be regarded as potentially serious Infections stimulate the natural defences of the body —the multiplication of phagocytic leukocytes and the synthesis of large amounts of protein antibodies Both processes require aminoacids derived from breakdown of storage proteins such as albumin (63) The rate of breakdown of storage protein is likely to exceed the difference between dietary intake and the body's synthetic requirements and in patients with M S U D who cannot catabolise the excess branched chain aminoacids a build up of aminoacids and their 2-oxoacids in the system results This accumulation of aminoacids and the associated acidosis will impair the body's synthetic and metabolic abilities thus completing a vicious circle and allowing the infection to gain a firm and potentially overwhelming hold on the patient

It is therefore of first importance that even mild infections are treated promptly and vigorously Appropriate antibiotic therapy total withdrawal of leucine isoleucine and valine or initially of all protein and immediate correction of any tendency towards systemic acidosis are of crucial importance Frequent plasma aminoacid analyses are again required to determine at what stage any or all of the branched chain aminoacids should be restored to the diet The time pattern for restoration of the individual aminoacids generally follows that of the initial therapy (Tables 1 *a* and *b*)

Finally a patient's requirements for branched chain aminoacids may alter rapidly depending upon the state of general health and previous nutrition It has been found ill advised to extend the interval between plasma aminoacid analyses beyond about three weeks and a critical reassessment of all dietary requirements aminoacids calories vitamin and minerals should be undertaken every three months Our experience shows that a sudden increase of plasma concentrations of branched chain aminoacids after period of good dietary control is of value as a reliable forewarning of infection in advance of the appearance of detectable clinical signs

## SUMMARY

An infant with Maple Syrup Urine Disease was treated from six weeks of age with a synthetic diet containing carefully restricted quantities of branched chain aminoacids There was a marked immediate improvement At twelve weeks gross vitamin deficiency developed and was corrected The patient is now more than four and a half years old and although in reasonable general health is quite severely retarded both mentally and physically

The problems of diagnosis the biochemical basis of dietary treatment and the laboratory requirements for control are discussed in relation to the 55 previously published cases

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## ADDENDUM

Since the preparation of this manuscript the patient contracted a respiratory tract infection there followed a rapid clinical and biochemical deterioration despite antibiotic fluid and alkali therapy She died on 24th November 1968 Macroscopic findings at autopsy were limited to bronchopneumonia detailed pathology and histochemistry will be the subject of a further report

branched chain 2-oxoacids is reversible and does not distinguish markedly between D and L 3 methyl 2-oxovaleric acid Furthermore the renal reabsorption mechanism does not distinguish isoleucine and allo isoleucine but ion exchange chromatography of acid hydrolysates of total plasma proteins and of haemolysates from our patient shows that there is no significant incorporation of L allo isoleucine into protein The result is not unexpected and rules out the possibility that the secondary metabolic disturbances of M S U D in particular the anaemia observed in our patient could have been due to the erroneous synthesis of inactive proteins containing allo isoleucine in agreement with Westall s independent findings (71)

*Initial response to dietary therapy*

During the first week of treatment the plasma concentrations of valine leucine and isoleucine fall precipitately and the 2 oxoacids disappear from the urine indicating a relief of the cause of the metabolic acidosis During this period liberal fluid intake is desirable administered if necessary by intravenous infusions untreated acidosis should be corrected and electrolyte balance should be subject to careful control Exchange transfusion is not warranted (21 63) in view of the very large amounts of the aminoacids stored in the tissues (see 65 and 18 for detailed figures) Indeed leucine may be concentrated to a stage where crystallization takes place within cells (29)

A period of two days during which no protein is fed to the patient does not seem in any way deleterious and allows time for the materials for dietary therapy to be acquired Pure synthetic L-aminoacids may be used but commercial aminoacid mixtures for the primary treatment and subsequent maintenance of patients with M S U D are available (from Scientific Hospital Supplies Ltd 38 Queensland Road, Liverpool 7) (Table 3) or may be prepared (12) Such mixtures contain none of the branched chain aminoacids, but a generous allowance of other essential aminoacids (19) and sufficient non essential aminoacids to make up the normal nitrogen requirements Calorie requirements are made up with corn oil arachis oil or fresh cream and sucrose or corn flour starch After not more than two days mineral and vitamin supplements as detailed by Dent & Westall (12) should be introduced

A second specimen of blood for plasma aminoacid analysis need not be taken until five days after the beginning of treatment Thereafter analyses every one or two days are desirable until the concentrations of branched chain aminoacids have all been reduced to normal after which analysis every four days to one week should be performed until dietary regulation has been established Where such frequent analyses are not possible or when the interval between sampling and analysis is greater than one day a great deal of intuition and interpolation must perforce be used As can be seen from the case history presented above the concentrations of the three branched chain aminoacids do not simultaneously return to normal (Table 1 *a*) Once the concentration of one aminoacid has returned to normal, however supplements of that aminoacid must be given to prevent a deficiency which would provoke the breakdown of body proteins with the concomitant production of large amounts of the branched chain aminoacids which cannot be catabolised Isolated observations suggest that this aspect is important (49 64)

*Experience with long term therapy*

The initial therapy and determination of requirements for maintenance must inevitably be undertaken on a completely empirical ad hoc basis the long term dietary treatment cannot be undertaken in any other way However our present experience leads us to make three general points

Firstly the requirements for the branched chain aminoacids decrease with age The requirements for leucine isoleucine and valine respectively in our case were 120 60 and 9[illegible] mg/kg per day at three months of age 75 4[illegible] and 60 at one year and at two years of age were 45 25 and 35 mg/kg per day This pa[illegible]

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# ANTIBODY DEFICIENCY SYNDROME AND AUTOIMMUNE HAEMOLYTIC ANAEMIA IN A BOY WITH ISOLATED IgM DEFICIENCY DYSIMMUNOGLOBULINAEMIA TYPE 5

G B A STOELINGA P J J van MUNSTER and J P SLOOFF

*From the Department of Pediatrics University of Nijmegen The Netherlands*

As our knowledge of the structure synthesis and function of immunoglobulins increases, attention is being focused increasingly on syndromes which entail a disturbance in specific antibodies

There are a great number of immunological deficiency diseases with antibody deficiency syndromes (16 16 30) one of these is dysgammaglobulinaemia (31) or dysimmunoglobulinaemia A classification of the latter is given in Table 1 Patients whose serum lacks only IgM are suffering from dysimmunoglobulinaemia type 5

Table 1 *Classification of dysimmunoglobulinaemias according to Stoelinga (34) which is virtually the same as that according to Hobbs (20) The classification adheres to the chronological order in which the syndromes were first described*

| Type | Absent immunoglobulin(s) | Immunoglobulins present in normal or increased amounts |
|---|---|---|
| 1 | IgA and IgM | IgG |
| 2 | IgG and IgA | IgM |
| 3 | IgG | IgA and IgM |
| 4 | IgA | IgG and IgM |
| 5 | IgM | IgG and IgA |
| 6 | IgG and IgM | IgA |

* Since little is known about the significance of the recently discovered IgD and IgE immunoglobulins in this context they will not be taken into account in this paper

Few patients with an isolated IgM deficiency have so far been described Schaller et al (32) mentioned this deficiency in a female infant who died at the age of 6 months, but the child had thymic hypoplasia as well Chapt et al (7) were unable to demonstrate IgM in immuno electrophoresis in a boy with a Wiskott–Aldrich syndrome who died from thymosarcoma Hobbs et al (19) described two children who died from meningococcosis and whose sera showed very low IgM concentrations Since Holborow (21) stated that Correlation of structure with function in antibody molecules had yet a long way to go indeed at present many properties of antibodies are more readily perceived than explained we deem it important to describe a patient with an antibody deficiency syndrome who later developed autoimmune haemolytic anaemia and in whom the serum had a very low IgM concentration An attempt will be made to correlate this deficiency with the inability to synthesize certain antibodies in order to enhance our understanding of the significance of IgM for immunological defence

## CASE REPORT

Patient P K was a boy born on 20th August 1955 as the fifth of six children From the first year of life he suffered from severe recurrent pneumonia with

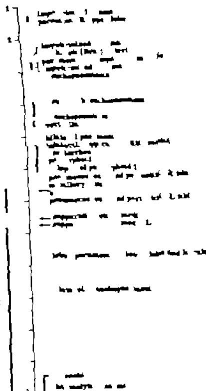


*Fig 1* Diagram showing infections which occurred in a patient with dysimmunoglobulinaemia type 5 also indicating therapy

extensive radiological changes In addition he had a chronic eczema with impetiginization on a few occasions He was repeatedly hospitalized for this condition (Fig 1) Bilateral otitis media bilateral pneumonia and steatorrhoea were diagnosed at age 5 Since the pneumonia was refractory to antibiotics and the steatorrhoea to pancreatic extracts and in view of marked deterioration of the general condition the boy was hospitalized for an exhaustive examination

On 19th January 1961 we saw a small, thin boy (height 108 cm weight 15 kg) with a dry skin and lichenified eczema of the wrists elbow folds axillae inguinal regions lower abdomen and popliteal fossae (In the course of th years this eczema has spread, and many large verrucae vulgares are always in evidence at the above mentioned sites and elsewhere on the skin) The tonsils were large but not distinctly inflamed there was bilateral suppurative otitis media. The heart was normal An infiltrate was demonstrable in the posterior part of the right lower lobe of the lungs The abdomen was distended due to meteorism but the abdominal organs showed no abnormalities on physical examination

Laboratory *findings* ESR 56/79 Hb 10 8 g/100 ml red cell count 3 22 $10^6$ haematocrit 33 4%

reticulocytes 12 per mille White cell count 12 000 with 3 % eosinophils 8 % staff cells 69 % segmented cells, 14 % lymphocytes and 6 % monocytes In the course of subsequent years the blood count never showed gross abnormalities apart from periods of infection the lymphocyte percentage varied between 20 and 40 % There were always many eosinophils (sometimes as many as 25 %) The platelet count was 658 000 and subsequently varied between 200 000 and 400 000

Serum electrolytes were always normal and liver functions undisturbed Total protein 58 5 g/l albumin 19 0 g/l $\alpha$ globulin 2 7 g/l $\alpha_2$-globulin 5 8 g/l $\beta$ and $\gamma$ globulins together 31 9 g/l

The faeces contained many fatty acids and undegraded fats the fat absorption coefficient was 65 % The Na concentration in sweat was 30 mEq/l Normal amounts of pancreatic enzymes were demonstrated in duodenal juice Urinalysis was normal

*Radiological* aspects of the gastrointestinal tract were consistent with those observed in coeliacia.

*Treatment* with antibiotics and a gluten free diet led to adequate improvement the fat absorption coefficient was increased to 80 %

Signs of pneumonia in both lower lobes and bilateral otorrhoea recurred in May 1961 An antrotomy disclosed no pus but the otitis media was subsequently cured.

In July 1961 the patient developed severe enteritis caused by Salmonella E (type Give) which led to shock and necessitated chloramphenicol medication and intravenous fluids. The symptoms recurred several times until a clinical cure was obtained salmonella remained long demonstrable in the faeces.

On 5th February 1962 the boy was hospitalized with fever and pain in the left leg periostitis of the left tibia was diagnosed and pneumococci were cultured from the blood. The symptoms were rapidly controlled by treatment with large doses of penicillin

Although treatment with *10 ml* $\gamma$-globulin 16 % per 4 weeks was started, pneumococcaemia with periostitis this time of the right tibia, recurred in June *196.* Again the condition responded rapidly to antibiotic medication After discontinuation of the $\gamma$-globulin therapy the patient again developed otitis me*dia* and symptoms of upper respiratory infection.

From June 1963 on he received 12 later 16 ml $\gamma$ globulin 16 % per 4 weeks and 500 mg sulphadiazine *daily* few infections have since occurred (cf. Fig 1) the boy was in excellent condition and height and weight were normal There were no signs of steatorrhoea but the extensive eczema persisted with many verrucae vulgares at the eczematous sites and elsewhere

Bronchography in June 1964 disclosed no bronchiectases

On 9th February 1968 the patient was hospitalized again after 14 days of fever and occasional slight jaundice

At examination he was found to be moderately ill pale and slightly jaundiced The skin changes were as before a fair number of lymph glands were palp-

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| Type | Absent immunoglobulin(s) | Immunoglobulins present in normal or increased amounts |
|---|---|---|
| 1 | IgA and IgM | IgG |
| 2 | IgG and IgA | IgM |
| 3 | IgG | IgA and IgM |
| 4 | IgA | IgG and IgM |
| 5 | IgM | IgG and IgA |
| 6 | IgG and IgM | IgA |

Since little is known about the significance of the recently discovered IgD and IgE immunoglobulins in this context they will not be taken into account in this paper

Few patients with an isolated IgM deficiency have so far been described Schaller *et al* (32) mentioned this deficiency in a female infant who died at the age of 6 months but this child had thymic hypoplasia as well Chaptal *et al* (7) were unable to demonstrate IgM at immuno-electrophoresis in a boy with a Wiskott–Aldrich syndrome who died from lymphosarcoma Hobbs *et al* (19) described two children who died from meningococcosis and whose sera showed very low IgM concentrations Since Holborow (21) stated that Correlation of structure with function in antibody molecules had yet a long way to go indeed at present many properties of antibodies are more readily perceived than explained we deem it important to describe a patient with an antibody deficiency syndrome who later developed autoimmune haemolytic anaemia and in whom the serum had a very low IgM concentration An attempt will be made to correlate this deficiency with the inability to synthesize certain antibodies, in order to enhance our understanding of the significance of IgM for immunological defence

## CASE REPORT

Patient P K was a boy born on 20th August 1955 as the fifth of six children From the first year of life be suffered from severe recurrent pneumonia with

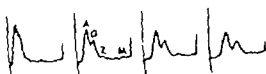



Fig 3 Ultracentrifugation of patient's serum at 9 780 rpm exposures 10 24 35 and 53 minutes after attaining this speed *A* = albumin $S_{20}$ = 3.8 S *Z* atypical macroglobulin $S_{20}$ = 9.3 S *G* = globulin S 6.6 S *M* macroglobulin $S_{20}$ = 16.5 S

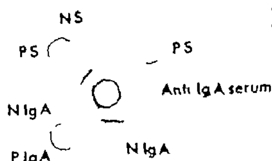



Fig 4 Ouchterlony study demonstrating immunological identity of normal IgA (N IgA) and patient IgA (P IgA) with anti α chain antiserum

ber (25) employing H chain specific antisera yielded the following values

| | Patient | Normal adult value |
|---|---|---|
| IgG | 1250 mg 00 ml | 1182 mg 100 ml SD 319 mg/100 ml |
| IgA | 550 mg 100 ml | 182 mg 100 ml SD 57 mg/100 ml |
| IgM | 6 mg 100 ml | 94 mg/100 ml, SD 76 mg/100 ml |

Although the precipitation line in immunoelectrophoresis was of normal appearance the greatly increased IgA concentration was suggestive of a paraprotein

Ultracentrifugation disclosed an atypical macroglobulin with $S_{20}$ = 9 3 S (Fig 3) However it is known (1) that a proportion of the serum IgA can have a sedimentation exceeding 7S as the IgA increases this fraction too can increase and become visible in the ultracentrifuge diagram as an additional peak In an attempt at definite elimination of an abnormality of the patient's IgA this was isolated from the patient's serum (according to Van Munster & Stoelinga (25)) and compared with normal serum IgA by the Ouchterlony technique a reaction of identity was demonstrated (Fig 4) with anti α-chain antiserum The isolated IgA contained both kappa and lambda chains

The above findings—some increase of IgG a distinct increase of IgA and very marked IgM deficiency—are conclusive of dysimmunoglobulinaemia In the absence of indications of structural abnormalities in IgG and IgA it was on the significance of the marked IgM deficiency that our study had to focus In this context it can be pointed out that the IgM concentration did not increase even after an acute infection or after vaccination

## *Bacteriology*

Haemolytic Staphylococcus aureus was isolated several times from the otorrhoeic pus and from sputum In addition pneumococci were repeatedly isolated from sputum There were likewise pneumococci in the blood on two occasions an effort at typing was futile the first time but type 6 was identified the second time Type 19 pneumococci were then isolated from the otorrhoeic pus The Salmonella which caused a severe enteritis was identified as species E group Five

## *Antibody formation*

The data on antibody formation collected before γ globulin therapy was instituted are summarized in Table 2

The following remarks may be made

1 Anti A and anti B isohaemagglutinins (the boy's blood group was 0/cdde) were virtually absent The technique used disclosed titres of 1 64 and 1 256 at this age we found equally low titres only in two patients with Bruton agammaglobulinaemia

2 The study of Bordetella pertussis was focused on capsular antigens against which no antibodies were produced

3 The capsular swelling method of Neufeld demonstrated no antibodies against type 6 and type 19 pneumococcal strains isolated after infections involving these strains The haemag

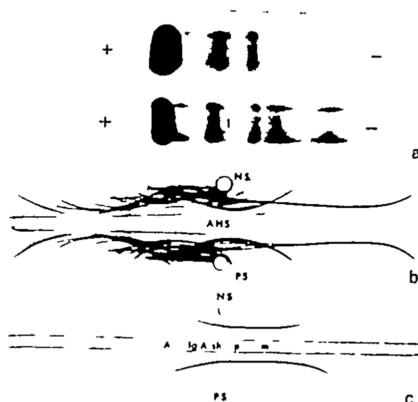



*Fig 2* (*a*) Agar-electrophoresis of normal serum (top) and patient's serum (bottom) (*b*) Immuno-electrophoresis with anti human serum (AHS) Note the absence of IgM precipitation (*c*) Immuno-electrophoresis with sheep anti IgA serum (NS = normal se... PS = patient's serum)

able in the cervical and inguinal regions No otitis media no tonsillitis Many scattered bronchitic murmurs were audible over the lungs and the X ray revealed a bronchitic pattern Liver and spleen were palpable about 4 cm below the costal margin and felt firm

*Laboratory findings* ESR 108/112 Hb 6 2 g/100 ml red cell count 1 8 10 haematocrit 18 5 reticulocytes 209 per mille white cell count 22 300 with 1 staff cells 67 segmented cells 8 eosinophils 20 lymphocytes and 4 monocytes

Serum electrolytes normal Bilirubin 3 75 mg/100 ml 10 minute percentage 37 4 alkaline phosphatases 44 2 U (Bodansky) thymol turbidity 2 U SGOT 47 U SGPT 8 U serum iron 241 g 199 ml binding capacity 286 g/100 ml saturation 84 Total protein 67 5 g/l albumin 41 6 g l α globulin 3 3 g l α globulin 5 2 g/l β globulin 3 4 g/l γ globulin 14 0 g/l The Coombs test was positive (cf special studies)

The urine contained much urobilin and a trace of bilirubin

The blood culture was sterile

After careful selection of various donors the boy received 1 l blood in 36 hours This raised the Hb to 10 6 g/100 ml haematocrit 28 5 Tolerance to the transfusions was good The Hb subsequently showed a gradual increase to 13 4 g/100 ml haematocrit 36 reticulocytes remained at about 70 per mille Additional medication included antibiotics and 2 5 mg prednisone The Hb thereupon remained constant and liver and spleen gradually diminished in size The Coombs test however remained positive

## SPECIAL STUDIES

### *Serum proteins*

The serum protein pattern remained constant through the years and did not change even during the period of haemolytic anaemia The total protein value as a rule was increased (70–85 g/l) this was caused by the high γ globulin concentration which ranged from 15 to 25 g/l Only during periods of servere infection did the albumin concentration diminish and therefore the total protein value while the γ globulin concentration remained unchanged At paper and agar electrophoresis (Fig 2 *a*) a high PAS positive band was visible in borderline region between β and γ globulin The immuno electrophoretic pattern was likewise constant There was an increase of IgG in the slow migration zone and a very pronounced increase of IgA (Figs 2 *b* and 2 *c*) IgM was invisible IgD was not demonstrable with specific antiserum (but with the serum we used it is impossible to visualize lower concentrations)

Quantitations of immunoglobulins by single radial immunodiffusion according to Mancini *et al* (23) with a modification described ear

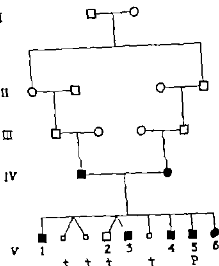



*Fig 5* Patient's family □ = male not examined ■ = male examined ○ = female not examined, ● = female examined ▫ premature birth *P* = proband




*Fig 6* Paper-electrophoresis of the deceased brother serum

fect of the leucocytes on pneumococci (according to 6) was completely intact

The lymph glands showed a macroscopically normal reaction to local inflammations the tonsils too were enlarged and inflamed on a few occasions The chest X ray showed a thymus shadow However we were unable to study the macroscopic aspect of a lymph gland after biopsy because the accessible lymph glands were localized in an area of extensive and readily impetiginized eczema a diagnostic intervention entailing such a risk of extensive infection seemed unjustifiable

The bone marrow showed an increased number of reticulocytes and megakaryocytes Neither the red nor the white system showed abnormalities There were many plasma cells of varying size The large cells had a light coloured protoplasm and sometimes a pyknotic nucleus the number of binucleate plasma cells was relatively large We gained the impression of considerable activity of the plasmacellular system

*Family*

The parents were consanguineous (Fig 5) The mother was the 4th of 18 and the father the 3rd of 6 children many of whom have children of their own In this large family there were no patients with an antibody deficiency syndrome or autoimmune disease Our patient was the 5th of 6 children 3 additional pregnancies ended in premature birth The 2nd child in this family (one of a pair of dizygotic twins) suffered a large number of infections (Fig 5 V–2)

He was born in 1951 and as an infant showed eczema with pyoderma An extensive eruption of pox occurred following vaccination Subsequently he developed pertussis varicella, infectious hepatitis measles and poliomyelitis in succession with intercurrent and subsequent extensive recurrent pneumonias on the basis of bronchiectases from which pneumococci were isolated Also there were frequent abscesses of the skin and lymph glands caused by Staphylococcus aureus

In 1956 he developed meningitis caused by Haemophilus influenzae and in 1957 he died from pneumococcal meningitis

This child's protein pattern likewise showed a high $\gamma$ globulin concentration, and the same band in the $\beta$ zone (Fig 6) as our patient (Fig 2) An exhaustive study of the serum proteins was not feasible at the time

Many plasma cells were found in the bone marrow and at post mortem in the peribronchial inflammatory tissue the lymph glands showed no abnormalities at microscopic examinations

The following immunoglobulin and isohaemagglutinin values were found in the parents

Table 2 *Antibody formation after infection or vaccination in a patient with dysimmunoglobulinaemia type 5*

| Typhoid Paratyphoid | | Other antibodies | | |
|---|---|---|---|---|
| anti O | anti H | | Before Booster | After Booster |
| Typhoid after vaccination | | Tetanus antitoxin | 0 001 AU/ml | >40 AU/ml |
| Vi 0 | | Diphtheria antitoxin | <0 01 AU/ml | 1 6 AU/ml |
| 9 12 0 | | Pertussis agglutination | <1 2 | 1 2 |
| | d 1 40 | Poliomyelitis type I | 512 | 256 |
| Salmonella (Gr e) after infection | | type II | 32 | 64 |
| 3 10 0 | | type III | 4 | 4 |
| | *b v 0* | *Antistaphylolysin* | 0 5 AU/ml | |
| | 1 7 1 20 | Antistaphylocoagulase | negative | |
| | | Antistreptolysin titre | 600 U | |
| Isohaemagglutinins | | Practically no antibody formation against isolated type 6 and type 19 pneumococci | | |
| anti A | anti B | Complement binding influenza A | negative | |
| | | Complement binding adenovirus | 1 320 | |
| <1 2 | 1 2 | Neutralization reaction to adenovirus *3 7 and 21* | negative | |
| | | Latex fixation test<br>Rose test<br>Wassermann reaction<br>Paul-Bunnell reaction<br>VDRL<br>ANF | negative | |

glutination technique employed by Stoop (35) revealed only a titre of 1 2

4 The absence of antibodies against influenza A has little significance because the boy may not have been in contact with the antigen

5 *Total complement was present* (titre 1 60 normal value 1 60–1 100) The complement component $\beta_1C$ was always demonstrable at immuno electrophoresis even in the acute haemolytic phase Properdine was not demonstrable in the serum but the method used sometimes fails to demonstrate this in normal serum also (Pondman personal communication)

*Autoimmune haemolytic anaemia*

When the blood was cross matched with that from a large number of donors (blood group O Rh−) the patient s serum proved to agglutinate all donor erythrocytes in varying degrees Testing on a panel of erythrocytes of different blood groups disclosed no specific activity against a given blood group antigen The serum contained cold auto panagglutinins (titres 1 16 at 4 C, 1 2 at 20 C, 1 1 at 37 C) The direct Coombs test was positive with anti IgG serum but negative with anti IgM anti IgA and with anti IgD serum The patient s erythrocytes agglutinated also with *anti complement serum*

After filtration on Sephadex G200 the antibodies were found to be contained not in the macoglobulin but in the 7S globulin fraction

The conclusion is that the serum as well as an eluate of the erythrocytes contained incomplete warm autoantibodies of the IgG type No specificity was demonstrable but they may be active against a factor in the Rhesus system The Hb concentration remained constant after the blood transfusions and during prednisone medication but the Coombs test was positive thereafter until now

*Cellular factors*

The inflammatory reaction of the skin according to Rebuck & Crowley s open window method (29) was normal The phagocytizing ef

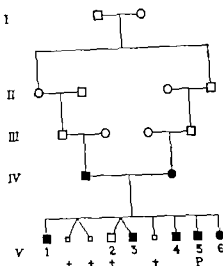



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| | Father | Mother |
|---|---|---|
| IgG | 800 mg/100 ml | 1100 mg/100 ml |
| IgA | 146 mg/100 ml | 375 mg/100 ml |
| IgM | 30 mg/100 ml | 220 mg/100 ml |
| anti A isohaemagglutinin | 1 32 | 1 126 |
| anti B isohaemagglutinin | 1 16 | 1 32 |

In view of the low paternal IgM concentration the high maternal IgA concentration, the parental consanguineousness and the death of a child from an antibody deficiency syndrome it seems highly probable that our patient was suffering from a familial congenital anomaly

## DISCUSSION

The clinical manifestations of recurrent severe infections in this boy were quite consistent with the syndrome described by such authors as Barandun *et al* (2) and Good *et al* (17) in association with disturbances in the defence mechanism An investigation into the cause of these disturbances in the defence mechanism disclosed no abnormalities other than changes in the immunoglobulin concentrations the serum IgG level was invariably above normal the IgA was in fact markedly increased and the IgM was absent or present only in a very low concentration The marked increase in IgA suggested the possibility of a paraprotein but this possibility was eliminated by ultracentrifugation and immunological techniques Consequently it seemed certain that the recurrent infections were related to the very low IgM concentration and in fact we never observed any increase of the serum IgM in response to an acute infection or vaccination

Apparently there was only a partly disturbed immunological defence mechanism in this case IgG and IgA were produced in sufficient amounts and antibodies were formed against certain antigens (tetanus diphtheria strepto cocci poliomyelitis) The lymph gland reacted normally to local inflammations and the tonsils too were occasionally distinctly enlarged and inflamed the bone marrow moreover always contained many plasma cells The clinical course was quite different from that observed in patients with disturbed thymus development Apart from the autoimmune haemolytic anaemia there were no indications of a disturbance in cellular immunity It must therefore be assumed that only IgM synthesis was disturbed in this boy and because certain specific antibodies were indeed formed in the other immunoglobulins it seems likely that there was a selective defect in the synthesis of the H$\mu$ polypeptide chains The family study supports the theory of a congenital disturbance in immunological defence The parents were consanguineous and a brother died with symptoms of an antibody deficiency syndrome in the brother too the infections were caused by Haemophilus influenzae pneumococci and haemolytic Staphylococcus aureus Althogh no immuno electrophoresis was feasible at the time the serum electrophoretic pattern in this brother closely resembled that in our patient an increase in the amount of protein in the $\beta$ zone Another striking finding was that the father repeatedly showed a low IgM concentration while the mother always had a high IgA concentration The immunoglobulin pattern in the remaining children was always normal We therefore believe that our patient was suffering from a familial congenital disturbance in IgM synthesis

A study of antibody formation (Table 2) showed that the isohaemagglutinins were virtually absent that no antibodies were formed against the capsular antigens of Haemophilus pertussis Haemophilus influenzae and pneumococci that no antibody formation occurred even after stimulation with Salmonella 0 antigens Some antibody formation against the flagellar (H) antigens did occur the antistreptolysin titre rose to 600 U after a streptococcal infection and antibodies were formed in normal quantities against diphtheria and tetanus toxin and the viral antigens which were investigated

It is generally assumed that the isohaemagglutinins and the antibodies against the somatic (0) Salmonella antigens are largely IgM antibodies (3 24) and the antibodies against the

saldr antigens of pneumococci and Haemophilus influenzae as determined here are like e IgM antibodies (35) It is precisely these gens against which our patient fails to pro- ce antibodies This demonstrates a good correlation between the IgM deficiency and the rtial immunological incapacity in this case e constant presence of extensive verrucae lgares too may have been a consequence of inability to produce antibodies against the usative virus After all Goffe *et al* (14) and dkin (28) have demonstrated that the antibodies against the verrucal virus are likewise the most part IgM antibodies

Schaller *et a* (32) also described a female tient whose serum lacked IgM but contained nsiderable amounts of IgA and electrophoretically slow IgG There was no antibody rmation after stimulation with typhoid vacne diphtheria and bacteriophage Φ X 174 he serum contained no 19S isohaemagglutins but there were 7S IgG antibodies against ythrocytes This patient, however suffered om a disturbance in delayed hypersensitivity he did not respond to γ globulin therapy and he postmortem disclosed lymphoid hypoplasia nd dysplasia of the thymus In this case herefore a disturbance in antibody formation was accompanied by a defect in cellular immunity Hobbs *et al* (19) described two children aged 8 and 5 who died from meningococcosis and in whose sera virtually no IgM could be demonstrated during the acute phase These children had not previously shown conspicuously frequent illness there were no earlier data on IgM concentration and the low IgM values found may therefore have been dependent on the acute process However a *few* relatives who themselves showed no recurrent infections were found to have low IgM concentrations so that a familial condition may have been involved

In patients with the Wiskott–Aldrich syndrome (sex linked thrombocytopenia eczema recurrent pyogenic infections) the serum contains no isohaemagglutinins Chaptal *et al* (7) described a patient with this syndrome whose serum contained no demonstrable IgM however no exhaustive study was made of the antibody formation Immunoelectrophoresis has yielded varying results in the Wiskott Aldrich syndrome some authors report chiefly an increased IgA concentration (22 36) whereas others emphasize a decreased IgM concentration (4 7) All patients with this syndrome lack isohaemagglutinins (8) and delayed hypersensitivity is often disturbed Blaese *et al* (4) demonstrated some deficiency in the formation of a number of specific antibodies

The possibility of a Wiskott–Aldrich syndrome in our patient was considered however there was no thrombocytopenia and the IgM deficiency was much more pronounced than has ever been reported in this syndrome Blaese *et al* (4) believe that the low IgM concentration in the Wiskott Aldrich syndrome may be imputable to a disturbance in the afferent limb of immunity i e inability to produce antibodies against polysaccharide antigens Because these antibodies are chiefly contained in IgM the concentration of IgM will be diminished in the presence of such an inability In our patient, however virtually no IgM was demonstrable so that a primary disturbance in IgM synthesis seems much more probable in this case

All patients with dysimmunoglobulinaemia type 1 2 3 or 5 have been described as showing the antibody deficiency syndrome In none of these types are characteristic symptoms observed which could be used in differentiation In this context it is of no importance whether the serum IgG concentration is normal or even increased (types 1 and 5) or whether the IgG concentration is distinctly decreased while the IgM concentration is normal or increased (types 2 and 3) Our patient with type 5 and some patients with type 1 are undoubtedly capable of producing a number of specific antibodies on the other hand most patients with dysimmunoglobulinaemia type 2 are capable of forming specific antibodies which normally occur in IgM Even this intact ability to form certain specific antibodies does not influence

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certain specific antibodies does not influence

the nature and severity of the clinical manifestations This would seem to warrant the conclusion that the presence of IgM as well as IgG is an absolute prerequisite for complete immunological defence

Various investigations (3 26 27, 33) have demonstrated that primary stimulation with antigen is followed first by formation of IgM antibodies, and only later by formation of antibodies with IgG properties A second contact with the same antigen is again followed by IgM antibody formation, but the IgG molecules now appear much earlier and in much larger amounts than after the primary stimulation Even though according to Freeman & Stavitsky (11) it is possible that IgG and IgM antibodies occur *simultaneously upon primary infection* but that the activity of IgM antibodies is more readily demonstrable the fact remains that primary antibody formation always involves *IgM antibodies The question arises as* to whether the fact that patients lacking only IgM develop an antibody deficiency syndrome implies that a phase of IgM antibody production is absolutely necessary for the formation of a number of antibodies To phrase it differently that IgG antibodies against a number of antigens cannot be formed unless IgM antibodies are formed first This is supported by experience patients with an antibody deficiency syndrome can be successfully treated with a $\gamma$ globulin preparation which contains no IgM In this pooled IgG there might be antibodies which the patients themselves are unable to produce Both in agammaglobulinaemia and in dysimmunoglobulinaemia certain micro organisms are always predominant as pathogens (staphylococci pneumococci Haemophilus influenzae) If the IgM antibodies are active especially against the lipopolysaccharides in the capsules of these bacilli then they can be active also against particulate antigens such as bacilli If lysis occurs as a result of contact of these particulate antigens with IgM antibodies (possibly in the presence of complement) then IgG antibodies should be capable of neutralizing soluble antigens (21)

Ellis & Smith (10) in a study of the consequences of splenectomy in infants (who often develop severe infections caused by Haemophilus influenzae pneumococci, streptococci or Gramnegative enterobacilli) likewise attached great importance to IgM antibodies i e to opsonins

That IgM plays an important role in the formation of antibodies against a number of common antigens is apparent also from the fact that IgM synthesis starts immediately after birth whereas IgG antibody production does not start until after a few weeks (33 36)

We believe that the findings obtained in our patient warrant the conclusion that the possibility of IgM antibody synthesis is an absolute *prerequisite for a complete immunological de*fence against bacterial pathogens No definite conclusion can as yet be formed concerning the exact function of IgM in this defence

*Our patient was treated during a 5 year pe*riod with sulphadiazine and $\gamma$ globulin and remained virtually free from infections during this period Then, suddenly and for no apparent reason the boy developed symptoms of haemolytic anaemia (which proved to be caused by incomplete warm autoantibodies)

It is difficult to explain this development We know that rheumatoid arthritis and other autoimmune diseases have a higher than normal incidence in patients with agammaglobulinaemia (12) and the same applies to these patients relatives even though they themselves show no evidence of an antibody deficiency syndrome However no autoimmune diseases were reported in our patient s relatives

*Haemolytic anaemia with a positive* Coombs test has been observed in patients where all three immunoglobulins were low (37) dysimmunoglobulinaemia (31) and in a few patients with dysimmunoglobulinaemia who in addition showed signs of thymic aplasia (5 13 15 32)

Hinz & Boyer (18) described an adult woman with haemolytic anaemia whose serum lacked IgA showed a greatly diminished IgG concentration and an excess of IgM the autoantibodies were contained in the IgM This

oman however showed no infections and no other evidence of an immunological disorder. A similar case was described by Hobbs *et al* (20) but their patient did show an antibody deficiency syndrome.

The autoantibodies in our patient are contained in the IgG; the small amount of IgM in the serum shows no demonstrable antibody activity. At least for the moment we must assume that this patient, in addition to suffering from a familial congenital disturbance in the production of a large number of antibodies, is subject to a disturbance which affects another factor of the immunological mechanism: the recognition of "self"-determination.

Obviously the inability to produce certain antibodies has preceded the occurrence of the autoimmune reaction, but it is not certain whether this autoimmunization likewise results from the IgM deficiency. The lack of IgM may have caused a disturbance in immunological homoeostasis, thus giving rise to the autoimmune phenomenon. On the other hand it is possible that this severe immunological imbalance is based on a general disorder. This point may be clarified in future. Meanwhile, the prognosis of this autoimmune haemolytic anaemia does not seem to be particularly favourable (9).


SUMMARY

This paper describes a boy who suffered from severe recurrent infections from the first year of life. A brother with similar symptoms died from *meningococcal meningitis*. *Virtually no* IgM was demonstrable in the patient's serum and it is suggested that the antibody deficiency syndrome in this case was based on a *familial* congenital disturbance in IgM synthesis. A study of antibody formation disclosed that disturbances existed in the formation of antibodies assumed to be contained in IgM; other antibodies were produced in adequate amounts. The significance of these findings is discussed in some detail. The boy subsequently *developed* autoimmune haemolytic anaemia. The question whether this was caused by the IgM deficiency or whether the immunological imbalance was based on a general disorder must remain unanswered.



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the nature and severity of the clinical manifestations This would seem to warrant the conclusion that the presence of IgM as well as IgG is an absolute prerequisite for complete immunological defence

Various investigations (3 26 27 33) have demonstrated that primary stimulation with antigen is followed first by formation of IgM antibodies, and only later by formation of antibodies with IgG properties A second contact with the same antigen is again followed by IgM antibody formation but the IgG molecules now appear much earlier and in much larger amounts than after the primary stimulation Even though according to Freeman & Stavitsky (11) it is possible that IgG and IgM antibodies occur simultaneously upon primary infection but that the activity of IgM antibodies is more readily demonstrable the fact remains that primary antibody formation always involves IgM antibodies The question arises as to whether the fact that patients lacking only IgM develop an antibody deficiency syndrome implies that a phase of IgM antibody production is absolutely necessary for the formation of a number of antibodies To phrase it differently that IgG antibodies against a number of antigens cannot be formed unless IgM antibodies are formed first This is supported by experience patients with an antibody deficiency syndrome can be successfully treated with a $\gamma$ globulin preparation which contains no IgM In this pooled IgG there might be antibodies which the patients themselves are unable to produce Both in agammaglobulinaemia and in dysimmunoglobulinaemia certain micro organisms are always predominant as pathogens (staphylococci pneumococci Haemophilus influenzae) If the IgM antibodies are active especially against the lipopolysaccharides in the capsules of these bacilli then they can be active also against particulate antigens such as bacilli If lysis occurs as a result of contact of these particulate antigens with IgM antibodies (possibly in the presence of complement) then IgG antibodies should be capable of neutralizing soluble antigens (21)

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The present study was carried out for two reasons firstly to establish figures for the normal values for haemoglobin packed cell volume erythrocyte count MCV MCHC serum iron and transferrin in children of 3–6 years of age secondly to get an impression of the frequency of iron deficiency in a randomly selected series of Danish children in this age group In spite of a voluminous literature dealing with blood and serum values the normal values in children of 3–6 years of age have not been sufficiently established Most studies were performed several years ago using methods for haemoglobin measurements which are less exact than those available today Moreover many studies do not make it adequately clear whether children with iron deficiency have been excluded from the material In the present study efforts were made to exclude such cases of iron deficiency anaemia partly by determinations of serum iron and partly by estimation of the effect of a period of administration of supplemental iron on the blood values

## MATERIAL AND METHODS

The material consisted of 147 children born in the years 1961 to 1964 inclusive and attending 6 different kindergartens in Copenhagen Questionnaires were sent to the parents asking about the presence of any chronic or temporary disease and of any iron intake within 4 5 months before the investigation One child had received 1 2 tablets of an iron preparation 3–4 months before the investigation another child 1 tablet of an iron preparation 3–4 weeks before the investigation Both cases were included in the material Apart from recent episodes of common colds no children were found with any chronic or temporary diseases Consequently none of the children were excluded from the investigation for this reason Each child had an ESR determination done and those whose values were in excess of 15 mm in one hour (25 children in the first sampling 31 children in the second sampling) had a white cell count done as well Two children in the first sampling and one child in the second sampling had a white cell count of about 14 000 cells per µl but since all other values were normal they were included in the material The blood samples were all drawn from a cubital vein at the same time of the day The haemoglobin concentration was determined by photometry on diluted blood by the cyanhaemoglobin method according to The International Committee for Standardization in Haematology of the European Society of Haematology (8) The photometry was done by means of a Bausch and Lomb Spectronic 20 photometer The readings of the photometer were controlled as follows Diluted samples were read in two Zeiss PMQ spectrophotometers and the haemoglobin concentration (c in g/100 ml) were determined from

$$c = F \cdot E = \frac{M \cdot D \cdot 10^{-6}}{\varepsilon \cdot l} E$$

where $E$ = extinction $M$ = the molecular weight of haemoglobin = 64 458 D = the dilution Factor = 251 $\varepsilon$ = the millimolar extinction coefficient = 44 0 l = pathway in cuvette = 1 cm

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Table 2 *Mean volumes of packed red cells (ml/100 ml) in the different age groups*

| Own material | | | | | Mugrage & Andresen (Mean) | Guest (Mean) | Wintrobe (Mean) |
|---|---|---|---|---|---|---|---|
| Age yrs | No of cases | Mean | S D | Range | | | |
| 3 | 16 | 35 | ±2.00 | 34–41 | 39.61 | 36.6 | 36.0 |
| 4 | 38 | 40 | ±2.37 | 36–45 | 40.10 | 36.9 | 37.0 |
| 5 | 66 | 40 | ±2.33 | 36–47 | 39.93 | 37.2 | 37.0 |
| 6 | 27 | 41 | ±2.78 | 35–46 | 39.87 | – | 37.5 (6–10 yrs) |
| 3–6 | 147 | 40 | ±2.37 | 34–48 | | | |

Table 3 *Mean numbers of red blood cells ($10^6/\mu l$) in the different age groups*

| Own material | | | | | Mugrage & Andresen (Mean) | Guest (Mean) | Wintrobe (Mean) |
|---|---|---|---|---|---|---|---|
| Age (yrs) | No of cases | Mean | S D | Range | | | |
| 3 | 16 | 4.84 | ±0.32 | 4.27–5.54 | 4.44 | 4.69 | 4.5 |
| 4 | 38 | 4.89 | ±0.34 | 4.06–5.61 | 4.43 | 4.61 | 4.6±0.5 |
| 5 | 66 | 4.90 | ±0.33 | 4.05–5.53 | 4.40 | 4.65 | 4.6 |
| 6 | 27 | 4.75 | ±0.35 | 4.37–5.48 | 4.41 | — | 4.7 (6–10 yrs) |
| 3–6 | 147 | 4.85 | ±0.34 | 4.06–5.61 | | | |

Table 4 Mean MCV (fl) in the different age groups

| Own material | | | | | Mugrage & Andresen (Mean) | Guest (Mean) | Wintrobe (Mean) |
|---|---|---|---|---|---|---|---|
| Age (yrs) | No of cases | Mean | S D | Range | | | |
| 3 | 16 | 79.7 | ±5.13 | 63.4–89.0 | 89.2 | 78 | 80 |
| 4 | 38 | 81.3 | ±3.94 | 73.8–90.3 | 90.5 | 80 | 80 |
| 5 | 66 | 81.7 | ±3.65 | 74.5–91.0 | 90.7 | 80 | 80 |
| 6 | 27 | 82.9 | ±3.47 | 69.9–93.2 | 90.4 | – | 80 (6–10 yrs) |
| 3–6 | 147 | 81.4 | ±4.05 | 68.4–89.0 | | | |

*7 Comparison of the values before and after iron supplement*

In order to estimate whether the observed mean values represent normal values it is important to exclude even minor degrees of iron deficiency anaemia. The investigation was therefore repeated after a period of iron administration. Because of absence at the second collection of samples (cessation of parents consent of participation in the kindergartens or illness at the time of the second sampling) the material was reduced to 122 children. Due to irregular attendance at the kindergarten the total amount of iron received varied to some extent 760–1000 mg (4 children), 1000–2000 mg (38 children) and 2000–5000 mg (80 children). The children received the iron supplement during a period of 28–44 days (mean 36 days) from October to December 1967. This length of time is considered sufficient for revealing an iron deficiency anaemia, as the haemoglobin concentration in this condition will increase about 1.75 g/100 ml per week (Wintrobe (9)).

Table 1 *Mean haemoglobin concentration (g/100 ml) in the different age groups*

| Own material | | | | | | | |
|---|---|---|---|---|---|---|---|
| Age (yrs) | No of cases | Mean | S D | Range | Mugrage & Andresen (Mean) | Guest (Mean) | Wintrobe (Mean) |
| 3 | 16 | 13 0 | ±0 58 | 12 1–14 1 | 13 18 | 12 4 | 12 5 |
| 4 | 38 | 13 5 | ±0 79 | 11 7–15 4 | 13 43 | 12 4 | 12 6 |
| 5 | 66 | 13 8 | ±0 87 | 12 1–16 2 | 13 27 | 12 7 | 12 6 |
| 6 | 27 | 14 0 | ±0 81 | 11 9–15 6 | 13 34 | — | 12 9 (6–10 yrs |
| 3–6 | 147 | 13 6 | ±0 76 | 11 7–16 2 | | | |

tified cyanhaemoglobin content (British Drug House) Duplicate determinations deviating more than 0 3 g/ 100 ml were discarded and the assay repeated

The erythrocyte cell volume was determined with a Christ *microhaematocrit centrifuge using micro* tubes 1 5 mm in diameter and 75 mm long sealed with wax and centrifuged at 15 000 R P M for 3 min The packed cell volume was read immediately after centrifugation by use of a Hawksley Micro Haematocrit Reader In some cases one out of the three samples from each subject had to be discarded because of incomplete sealing or breach during centrifugation

The erythrocytes were counted in a cellescope (Coulter counter Model D Serie 2094 2/4 Ashwell St St Albans Herts) according to the recommendations of the manufactures For each series of determinations the setting of the instrument was controlled by parallel counts in conventional counting chambers (Fuchs Rosenthal) MCV and MCHC were calculated as usual according to Wintrobe (9) Serum iron was determined spectrophotometrically according to Peters *et al* (5) TIBC (transferrin) was assayed by adding iron as the ferrous ion removing unbound excess by Al O column chromatography and estimation the serum iron as quoted

The leucocytes were counted *in a Fuchs Rosenthal* counting chamber All dilutions were made with semi automatic dispensors and microcaps These pipettes consist of short lengths of precision made capillary glass tubing calibrated to contain a definite volume from end to end They are accurate to within 1 or less and are discarded after use Pipettes and dispensors were repeatedly controlled by weighing out All assays were done in duplicate from venous blood

The analytical error (standard deviation) was calculated from the *deviation of duplicate determinations* Maximal tolerance for duplicate determinations were observed Estimations exceeding the tolerance were repeated The standard deviations and tolerance *(in parentheses)* were as follows haemoglobin 0 1 (0 3) g/100 ml packed cell volume *0 7 (1 9)* (v/v) erythrocyte count 0 13 (0 36) mill/μl serum iron 3 (9) μg/100 ml *and transferrin 6 (17) μg/100 ml*

The iron supplement was given as tablets of ferrous glycine sulphate (Glycifer®) 120 mg Fe *daily* 5 days a week in the kindergartens controlled by its employees

Haemoglobin packed cell volume ESR and WCC determinations were performed at the laboratory of *Queen Louise's Children's Hospital* the erythrocytes were counted at the central laboratory of Finsens Institute and serum iron plus TIBC determinations were performed at the central laboratory of Bispebjerg hospitalet

## RESULTS

### *1 Initial Material*

The Tables 1–7 represent the blood values of 147 children investigated before supplemental iron was given The figures all compared to values of similar studies from the literature (? 4 6 7 9) The present material is divided in 4 age groups (3 4 5 and 6 years) *but not in* sex groups since the same results were found for both sexes the highest discrepancy between the values for boys and girls being insignificant ($0.2 < p > 0.5$)

*The total material* exhibited the following mean values haemoglobin 13 6 g/100 ml volume of packed red cells 40 ml/100 ml *red* blood cell count 4 85 mill/μl mean corpuscular volume 81 4 fl mean corpuscular haemoglobin concentration 34 1 g/100 ml serum iron 117 μg/100 ml and total iron binding capacity 402 μg/100 ml

An increase in the values from the age of 3 to 6 was found in the values of haemoglobin (13–14 g per 100 ml) V P R C (38–41 % v/v) and MCV (80–83 fl) A similar but smaller increase *in the values with the age is shown in the papers quoted in the tables*

Table 2 *Mean volumes of packed red cells (ml/100 ml) in the different age groups*

| Own material | | | | | Mugrage & Andresen (Mean) | Guest (Mean) | Wintrobe (Mean) |
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| 4 | 38 | 40 | ±2.37 | 36–48 | 40.10 | 36.9 | 37.0 |
| 5 | 66 | 40 | ±2.33 | 36–47 | 39.93 | 37.2 | 37.0 |
| 6 | 27 | 41 | ±2.78 | 35–46 | 39.87 | — | 37.5 (6–10 yrs) |
| 3–6 | 147 | 40 | ±2.37 | 34–48 | | | |

Table 3 *Mean numbers of red blood cells ($10^6/\mu l$) in the different age groups*

| Own material | | | | | Mugrage & Andresen (Mean) | Guest (Mean) | Wintrobe (Mean) |
|---|---|---|---|---|---|---|---|
| Age (yrs) | No of cases | Mean | S D | Range | | | |
| 3 | 16 | 4.84 | ±0.32 | 4.27–5.54 | 4.44 | 4.69 | 4.5 |
| 4 | 38 | 4.89 | ±0.34 | 4.06–5.61 | 4.43 | 4.61 | 4.6±0.5 |
| 5 | 66 | 4.90 | ±0.33 | 4.08–5.53 | 4.40 | 4.65 | 4.6 |
| 6 | 27 | 4.75 | ±0.35 | 4.37–5.48 | 4.41 | — | 4.7 (6–10 yrs) |
| 3–6 | 147 | 4.85 | ±0.34 | 4.06–5.61 | | | |

Table 4 *Mean MCV (fl) in the different age groups*

| Own material | | | | | Mugrage & Andresen (Mean) | Guest (Mean) | Wintrobe (Mean) |
|---|---|---|---|---|---|---|---|
| Age (yrs) | No of cases | Mean | S D | Range | | | |
| 3 | 16 | 79.7 | ±5.13 | 68.4–89.0 | 89.2 | 78 | 80 |
| 4 | 38 | 81.3 | ±3.94 | 73.8–90.3 | 90.5 | 80 | 80 |
| 5 | 66 | 81.7 | ±3.65 | 74.5–91.0 | 90.7 | 80 | 80 |
| 6 | 27 | 82.9 | +3.47 | 69.9–93.2 | 90.4 | — | 80 (6–10 yrs) |
| 3–6 | 147 | 81.4 | ±4.05 | 68.4–89.0 | | | |

*2 Comparison of the values before and after iron supplement*

In order to estimate whether the observed mean values represent normal values it is important to exclude even minor degrees of iron deficiency anaemia The investigation was therefore repeated after a period of iron administration Because of absence at the second collection of samples (cessation of parents consent, of participation in the kindergartens or illness at the time of the second sampling) the material was reduced to 122 children Due to irregular attendance at the kindergarten the total amount of iron received varied to some extent 760–1000 mg (4 children) 1000–2000 mg (38 children) and 2000–5000 mg (80 children) The children received the iron supplement during a period of 28–44 days (mean 36 days) from October to December 1967 This length of time is considered sufficient for revealing an iron deficiency anaemia, as the haemoglobin concentration in this condition will increase about 1.75 g/100 ml per week (Wintrobe (9))

Table 5 *Mean MCHC (g/100 ml) in the different age groups*

| Own material | | | | | Mugrage & Andresen | Guest | Wintrobe |
|---|---|---|---|---|---|---|---|
| Age (yrs) | No of cases | Mean | S D | Range | (Mean) | (Mean) | (Mean) |
| 3 | 16 | 33 6 | ±1 02 | 31 7–35 0 | 33 2 | 34 0 | 35 |
| 4 | 38 | 33 9 | ±1 39 | 31 0–36 0 | 33 4 | 33 7 | 34 |
| 5 | 66 | 34 4 | ±1 04 | 31 7–36 6 | 33 2 | 34 2 | 34 |
| 6 | 27 | 34 5 | ±1 41 | 32 6–39 6 | 33 4 | — | 34 (6–10 yrs) |
| 3–6 | 147 | 34 1 | ±1 22 | 31 0–39 6 | | | |

As the values of haemoglobin V P R C R B C MCHMC, serum iron and TIBC according to Table 8 were not influenced by the iron medication it seems justified to consider them as representing the normal values for the age group in question The significance of the difference was tested by means of the *t* test (1) The number of degrees of freedom was 12 in the age group of 3 24 in the age group of 6 and > 30 in the age group of 4 and 5 years

Table 6 *Mean concentrations of serum iron (μg/100 ml) in the different age groups*

| | Age (yrs) | No of cases | Mean | S D | Range |
|---|---|---|---|---|---|
| Own material | 3 | 15 | 110 | ±36 | 55–171 |
| | 4 | 38 | 111 | ±40 | 50–243 |
| | 5 | 65 | 122 | ±40 | 27–252 |
| | 6 | 23 | 123 | ±41 | 41–201 |
| | 3–6 | 141 | 117 | ±40 | 27–252 |
| Sturgeon | 3–10 | | 27–153 | | |
| | 6 | 86 | | | |
| Smith | 2–6 | | 116 | | |

Table 7 *Mean concentrations of TIBC (μg Fe/100 ml) in the different age groups*

| | Age (yrs) | No of cases | Mean | S D | Range |
|---|---|---|---|---|---|
| Own material | 3 | 14 | 396 | ±53 | 302–515 |
| | 4 | 32 | 419 | ±73 | 320–758 |
| | 5 | 59 | 411 | ±49 | 295–566 |
| | 6 | 23 | 381 | ±78 | 321–615 |
| | 3–6 | 128 | 402 | ±63 | 295–758 |
| Sturgeon | 3–10 | | 187–658 | | |
| | 6 | | 404 | | |
| Smith | 2–6 | | 395 | | |

The increase in MCV recorded in Table 8 c not be of any reality as a significant incr was not found in the values (V P R C R B C ) on which the calculation of MC based

In Fig 1 the frequency distribution of h moglobin before and after iron supplemen shown

## DISCUSSION

The mean values of the present study found to be equivalent or in most instanc higher than the corresponding figures given b previous investigators (2 3 4 6 7 9) Th haemoglobin content was 0 5–1 2 g/100 m higher than indicated in the American paper Different laboratory techniques can hardly b the only explanation because a correspondin increase in the packed cell volume was foun also Whether or not undiagnosed iron def ciency anaemia might have been included i the material of those studies remains impo sible to ascertain

*In the initial material there seems to be* tendency to increasing values of haemoglobi V P R C MCV and MCHC Although this in crease apparently was significant according to statistical methods (0 001 < $p$ > 0 05) it should be looked upon with some reservation on ac count of the relative small number in the single age groups even more as a similar increase was not found to the same extent in the iron treated group Of greater interest is therefore the mean values of the total group in this ma terial of 122 children haemoglobin (13 7–13 7

*Table 8. Mean values before (a) and after (b) supplementary iron in the different age groups*

| Age (yrs) | Haemoglobin (g/100 ml) a b | V P R C (ml/100 ml) a b | R B C ($10^6/\mu l$) a b | MCV (fl) a b | MCHC (g/100 ml) (R B C) a b | Fe (µg Fe/100 ml) a b | TIBC (µg Fe/100 ml) a b |
|---|---|---|---|---|---|---|---|
| 3 | 13.2 13.5<br>t 0.50<br>p n s | 39 39<br>t=0<br>p=n s | 4.97 4.71<br>t=0.28<br>p=n s | 79.5 83.6<br>t=3.13<br>p= <0.01 | 33.6 34.4<br>t=1.45<br>p=n s | 113 90<br>t=1.58<br>p=n s | 402 391<br>t=0.94<br>p=n s |
| 4 | 13.6 13.7<br>t=0.22<br>p=n s | 40 40<br>t 0<br>p=n s | 4.92 4.65<br>t=0.43<br>p=n s | 81.2 85.2<br>t=6.15<br>p= <0.001 | 33.9 34.6<br>t=2.00<br>p= <0.05 | 134 103<br>t=3.76<br>p= <0.001 | 428 391<br>t=2.43<br>p= <0.05 |
| 5 | 13.8 13.7<br>t=0.28<br>p=n s | 40 40<br>t=0<br>p=n s | 4.91 4.69<br>t=0.25<br>p=n s | 81.9 85.0<br>t=5.53<br>p= <0.001 | 34.3 34.5<br>t=0.91<br>p=n s | 119 114<br>t=0.77<br>p=n s | 406 405<br>t=0.10<br>p=n s |
| 6 | 14.0 13.8<br>t=0.53<br>p=n s | 40 40<br>t=0<br>p=n s | 4.91 4.64<br>t=0.19<br>p=n s | 82.5 86.1<br>t=3.30<br>p= <0.001 | 34.7 34.4<br>t=1.03<br>p=n s | 123 116<br>t=0.70<br>p=n s | 405 402<br>t=0.19<br>p=n s |
| Total Group | 13.7 13.7<br>t 0<br>p n s | 40 40<br>t=0<br>p=n s | 4.93 4.67<br>t=0.29<br>p=n s | 81.3 85.0<br>t=4.53<br>p= <0.001 | 34.1 34.5<br>t=1.35<br>p=n s | 122 106<br>t=1.45<br>p=n s | 410 397<br>t=0.92<br>p=n s |

g/100 ml) V P R C (40–40 ml/100 ml) R B C (4 93–4 67 mill/µl) MCV (81 3–85 0 fl) MCHC (34 1–34 5 g/100 ml) serum iron (122–106 µg/100 ml) and TIBC 410–397 µg/100 ml)

In order to ascertain whether the material of this study represents a normal cross section of the relevant age groups marginal values of haemoglobin serum iron and transferrin were analysed The highest values of haemoglobin did not indicate pathological conditions as none of the children had any disease which could lead to a secondary polycythaemia The lowest values of haemoglobin nine children with a haemoglobin content lower than 13 g/100 ml gave no evidence of any iron deficiency anaemia since the serum iron concentrations were found to be normal in each case and the values of MCV and MCHC were found not to deviate from the average values of the whole series

Two children before and two children after the iron supplement had serum iron concentrations generally agreed to be subnormal (27–37 µg 100 ml) In all four cases a normal concentration of transferrin and in two cases no definite decrease in the transferrin after the iron intake militate against iron deficiency

Neither could four cases in which the values of transferrin exceeded 500 µg/100 ml have been suffering from iron deficiency since they showed normal serum iron concentrations In this case as well as in one case where the transferrin concentration was normal before the

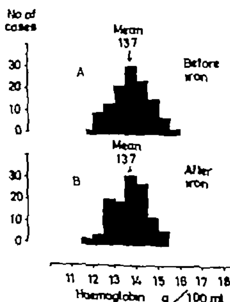



*Fig 1* Frequency distribution of haemoglobin values in 1_2 children 3–6 years of age before (*A*) and after (*B*) iron supplement

iron supplement and showed a higher value after the iron intake a reasonable explanation seems to be offered only by the uncertainty of the analysis

Due to the increased iron requirement of growth iron deficiency anaemia though most often found in the first two years should also be expected to appear to some extent in the following years In the present study however no cases of iron deficiency anaemia were found This seems to indicate that the frequency of iron deficiency anaemia is low in Denmark A reason for this could be that in addition to the prophylatic health examinations in the two first years of life a yearly health examination at the general practitioners is offered to all children between the age of two to seven These examinations include frequent haemoglobin estimations and children found with values lower than the average are usually treated with iron


## SUMMARY

Blood values including serum iron and transferrin were measured in 147 healthy children 3–6 years of age An iron supplement given to 122 children showed no significant changes in the mean values of the total material in haemoglobin (13 7–13 7 g/100 ml) V P R C (40–40 ml/100 ml) R B C (4 93–4 67 mill/$\mu$l) MCHC (34 1–34 5 g/100 ml) serum iron (122–106 $\mu$g/100 ml) and TIBC (410–397 $\mu$g/100 ml) These values are therefore considered as normal values for the age group in question No iron deficiency with or without anaemia was observed


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# CHILDHOOD HYPOGLYCAEMIA AS A SEQUEL OF ERYTHROBLASTOSIS FOETALIS

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Hypoglycaemia is the most frequent of the treatable metabolic causes of convulsions in childhood and early diagnosis is important because delay may lead to severe brain damage. The recognition that certain conditions in pregnancy are associated with an increased frequency of hypoglycaemia is clinically useful and has provided useful leads for research. The association of low birth weight for gestation (usually following maternal hypertension, toxaemia or renal disease) with neonatal hypoglycaemia is well known but it is less widely known that this type of baby may develop symptoms of hypoglycaemia later in childhood. Some individual babies may suffer hypoglycaemia in the newborn period and also as children, others have no symptoms of hypoglycaemia in the first week and present later in infancy or in childhood. These children develop hypoglycaemia on fasting with or without provocation by ketosis. (The less frequent children with leucine sensitive hypoglycaemia or with islet cell tumours have usually been of at least normal size at birth and some have been excessively large babies.)

Even before the cause of erythroblastosis foetalis was known, morbid anatomists had drawn attention to the occurrence of islet cell hyperplasia in the pancreas of babies who died of this disease (1). Clinical accompaniments of this finding have been described only recently in the form of neonatal hypoglycaemia (2, 3) and of hyperinsulinism (3) in babies with erythroblastosis.

This paper reports severe hypoglycaemia in two siblings with symptoms appearing 7 months and 25 months respectively after births which were complicated by very severe erythroblastosis. It is suggested that the hypoglycaemia represents a sequel of the erythroblastosis. The affected children were the fourth and fifth in a family of five children born to healthy unrelated parents. The third child died on the 12th day with a subarachnoid haemorrhage—the blood sugar was never measured.

## METHODS

Blood sugar estimations in Case 1 in 1962 were performed by the method of Asatoor & King (4). The remaining blood glucose levels were measured by a glucose oxidase method (5). Plasma insulin and growth hormone were estimated by radio-immunoassay using dextran coated charcoal (6, 7).

## FAMILY AND CASE HISTORIES

Mother. Born April 30, 19[illegible]4. Blood group A, Rhesus negative. Developed pulmonary tuberculosis April 1968.

Father. Born November 4, 1923. Blood group A, Rhesus positive. Healthy.

First child. Female, born January 21, 1957. Birth weight was [illegible]640 g at 43 weeks gestation. Healthy.

Second child. Female. Group A, Rhesus positive, born November 28th 1958 after spontaneous prema

Table 1 *Blood sugar levels in Case 1 over first 2 days in hospital*

| | Day 1 | | Day 2 | |
|---|---|---|---|---|
| Time | Feeding[a] | Blood sugar[b] (mg per 100 ml) | Feeding[a] & Drugs | Blood sugar[b] (mg per 100 ml) |
| 8 00 a m | 180 ml | | | |
| 9 00 a m | | | | 52 |
| 10 00 a m | | 28 | 180 ml | 43 |
| 11 00 a m | | 56 | | 24 |
| 12 00 MD | 90 ml | 80 | | 55 |
| 1 00 p m | | 73 | I M Glucagon 0 2 mg | |
| 2 00 p m | 90 ml | 43 | 180 ml | 131 |
| 3 00 p m | | 27 | | 110 |
| 4 00 p m | | 46 | | 36 |

[a] Unmodified cow s milk
[b] Method of Aratoor & King (4)

ture labour at 35 weeks gestation Birth weight was 2070 g Jaundice developed 8 hours after birth and the direct Coombs test was positive Exchange trans fusions were performed at 16 and 36 hours Subse quent progress has been normal

Third child Female group A Rhesus positive born August 8 1959 after induction of labour at 33 / weeks gestation Birth weight was 1530 g Cord haemoglobin level was 11 6 g per 100 ml Jaundice developed at 6 hours and exchange transfusions were performed at 14 40 and 90 hours of age She pro gressed very well until the 10th day when sudden loss of consciousness and circulatory collapse occurred Blood stained cerebrospinal fluid was obtained on lumbar puncture Blood sugar was not measured Death occurred on the 12th day

Necropsy revealed several areas of subarachnoid haemorrhage over the cerebellum which extended into the cortical tissue The pancreas was not examined microscopically

Fourth child (Case 1 M A N ) Female Group A Rhesus positive born January 26 1962 after in duction of labour at 35 weeks gestation Birth weight was 1200 g Cord blood haemoglobin level was 5[illegible] g per 100 ml Exchange transfusion was performed at 3 hours and jaundice was never severe (highest bilirubin level 19 9 mg per 100 ml on 9th day) so no further treatment was required Apnoeic attacks caused concern during the first 5 days of life but the blood sugar was not measured Solu Cortef had been given (10 mg every 6 hours) from birth because the platelet count was low Staphylococcal pneumo nia developed and was very slow to clear She made good progress from the age of 3 months until 7 months and was then sitting unaided and developing normally One morning two weeks later she was found unconscious and convulsing She was admitted to another hospital but continued to convulse almost constantly for 3 / weeks despite large doses of standard anticonvulsants and did not regain con sciousness Lumbar puncture yielded normal cerebro spinal fluid and X rays of the skull were normal

On transfer to the Royal Children s Hospital on October 12 1962 she was unconscious with increased muscle tone and frequent minor twitchings of the limbs Blood sugar (total reducing substances) was measured 8 times daily for 2 days Figures below or near 30 mg per 100 ml were obtained 4 times and these all occurred 1–2 hours after a milk feeding (Table 1) Leucine sensitive hypoglycaemia was su spected but the child s condition prevented formal testing Frequent feeding with 10 dextrose and the use of I M Glucagon 0 15–0 2 mg 3 hourly were given in the hope of saving her life and cerebral func tion Some decrease in twitching and a gradual slight improvement in consciousness occurred over a week on this regime but it became clear that gross brain damage was present and that full recovery was im possible Pneumoencephalography later revealed gross generalised dilatation of the ventricular system with thinning of the cerebral cortex Active treatment w

Table 2 *Oral glucose tolerance test results*

| | Case 1 (1 6 g/kg) | | | Case 2 (2 2 g/kg) | | |
|---|---|---|---|---|---|---|
| Time (min) | Blood glucose (mg per 100 ml) | Plasma insulin (microunits per ml) | Plasma growth hormone (millimicro grams per ml) | Blood glucose (mg per 100 ml) | Plasma insulin (microunits per ml) | Plasma growth hormone (millimic ro- grams per ml) |
| Fasting | 78 69 | 39 | 16 8 | 75 | 2 | 23 4 |
| 30 | 115 | 97 | | 136 | 2 | |
| 60 | | | | 124 | 6 | |
| 75 | 108 | 93 | | | | |
| 90 | | | | 167 | | |
| 120 | 106 | | 4 | 70 | | 9 |
| 180 | 70 | 70 | | 68 | <2 | |
| 240 | 17 | | 35 | 66 | | 28 |
| 300 | 24 | | 14 8 | 66 | | 16 4 |

Table 3 *Leucine tolerance test in Case 1*

0 15 g/kg orally

| Time (min) | Blood glucose (mg/100 ml) | Plasma insulin (microunits per ml) |
|---|---|---|
| Fasting | 82 | 6 |
| 0 | 67 | <5 |
| 40 | 62 | |
| 60 | 54 | |
| 90 | 60 | |
| 120 | 63 | |

therefore withdrawn At the request of the parents investigation was pursued no further

Tragically this child survived until the age of 6 / years. She could swallow some liquid from a spoon but showed no other functions After the occurrence of hypoglycaemia in Case 2 she was investigated more fully in May 1968 Glucose loading was followed by hypoglycaemia in the 4- and 5 hour specimens (Table 2) There was no abnormal sensitivity to leucine (Table 3) and an attempt to induce ketosis was abandoned after 7 days on a diet with a fatty acid to glucose ratio of 3 1 The level of immunoreactive insulin in her serum was high in one fasting specimen (Table 2) but not in a second (Table 3) the subsequent rise in this level after glucose was not excessive but the level remained very high at 1 0 minutes Plasma growth hormone levels were suppressed and rose again normally after the glucose load (Table 2)

She died in October 1969 of bronchopneumonia Autopsy revealed gross cerebral cortical atrophy and microscopic neuronal loss in the parietal and occipital regions The loss was maximal in the outer zones of the cortex but in the watershed areas all neurones were lost. The hippocampus cerebellum and basal ganglia showed microscopic damage The distribution of the neuronal loss was not quite typical for hypoglycaemic damage does not usually involve the visual cortex and hippocampus There was slight hyperplasia of the pancreatic islets and the β-cells showed some nuclear polyploidy No adenomata were found

Fifth child (Case P R N ) Male Group A Rhesus positive was born on April 5th 1966 after induction of labour at 34 weeks He was hydropic at birth and was not weighed Cord haemoglobin level was 9 g per 100 ml and a partial exchange transfusion was performed immediately followed by a normal exchange transfusion at 4 hours He progressed very well indeed after this and was never more than slightly jaundiced He weighed 1900 g at one week Physical and developmental progress were satisfactory At two months of age blood glucose estimations were performed after a 1 hour fast and hourly for four hours after a milk formula The results were all in the range 60-80 mg per 100 ml At 14 months some habit movements of the face occurred for two or three weeks An E.E.G was performed to reassure the parents and was normal Development was assessed as that of a 12-14 month old normal child Normal development continued until he was two years old At this time his mother developed pulmonary tuberculosis, and he had to be placed in a children's residential nursery He became rather withdrawn and frequently refused food Early one morning six weeks later he was found semi-conscious.

When admitted to the Royal Children's Hospital three hours later he was still semi-conscious and was pale and sweating with a rapid weak pulse The clinical diagnosis of hypoglycaemia was soon confirmed by a Dextrostix reading of less than 40 mg per 100 ml a blood glucose estimation of 17 mg per 100 ml and a very gratifying response to intravenous glucose infusion He regained consciousness in 10-20 minutes, and full normal alertness returned over 8 12 hours. When normal meals were started he was found to refuse everything other than milk and some carbohydrate foods Despite this he could sleep from 6 p m until 6 a m without developing hypoglycaemia. Dextrostix readings were always above 60 mg per 100 ml on awakening Consequently fasting from 10 p m to 10 a.m was considered safe as preparation for a glucose tolerance test On the morning of the test he awakened at 6 a m as usual and seemed well However he became upset when other children in the ward ate breakfast and by 9 a.m he was rather quiet and drowsy At 9 15 a m he convulsed and his blood glucose level was found to be 20 mg per 100 ml He responded quickly to intravenous glucose infusion No ketones were present in urine passed one hour later A glucose tolerance test was finally performed after six hours fasting and his blood glucose levels were normal up to five hours (Table ) Glucagon 0 5 mg caused no rise in blood glucose from a level of 28 mg per 100 ml but 1 0 mg caused a reasonable response from a level of 81 mg per 100 ml After the first of these tests another hypoglycaemic attack occurred No attempt was made to give a ketogenic diet because it was considered to be impossible to persuade him to eat the diet A leucine tolerance test was not performed because his sister had shown no sensitivity and because his hypoglycaemic attacks had only occurred after fasting

Plasma insulin levels were low (Table 2) and plasma growth hormone responded normally to a glucose load despite a slightly elevated fasting level

Management has aimed at increasing his protein intake and avoiding prolonged fasting except when asleep Some carbohydrate food is given as soon as he awakens in the morning No further attacks have occurred in the last 5 months

## DISCUSSION

The two children described both suffered severe erythroblastosis foetalis and both devel

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| 4 00 p m | | 46 | | 36 |

[a] Unmodified cow s milk
[b] Method of Asatoor & King (4)

ture labour at 35 weeks gestation Birth weight was 2070 g Jaundice developed 8 hours after birth and the direct Coombs test was positive Exchange transfusions were performed at 16 and 36 hours Subsequent progress has been normal

Third child Female group A Rhesus positive born August 8 1959 after induction of labour at 33 / weeks gestation Birth weight was 1530 g Cord haemoglobin level was 11 6 g per 100 ml Jaundice developed at 6 hours and exchange transfusions were performed at 14 40 and 90 hours of age She progressed very well until the 10th day when sudden loss of consciousness and circulatory collapse occurred Blood stained cerebrospinal fluid was obtained on lumbar puncture Blood sugar was not measured *Death occurred on the 12th day*

Necropsy revealed several areas of subarachnoid haemorrhage over the cerebellum which extended into the cortical tissue The pancreas was not examined microscopically

Fourth child (Case 1 M A N) Female Group A Rhesus positive born January 26 1962 after induction of labour at 35 weeks gestation Birth weight was 1200 g Cord blood haemoglobin level was 51 g per 100 ml Exchange transfusion was performed *at 3 hours* and jaundice was never severe (highest bilirubin level 19 9 mg per 100 ml on 9th day) so no further treatment was required Apnoeic attacks caused concern during the first 5 days of life but *the blood sugar was not measured Solu Cortef had* been given (10 mg every 6 hours) from birth because the platelet count was low Staphylococcal pneumonia developed and was very slow to clear She made *good progress from the age of 3 months* until 7 months and was then sitting unaided and developing normally One morning two weeks later she was found unconscious and convulsing She was admitted to another hospital but continued to convulse almost constantly for 3 / weeks despite large doses of standard anticonvulsants and did not regain consciousness Lumbar puncture yielded normal cerebrospinal fluid and X rays of the skull were normal

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Table 2 *Oral glucose tolerance test results*

| | Case 1 (1 6 g/kg) | | | Case 2 (2 2 g/kg) | | |
|---|---|---|---|---|---|---|
| Time (min) | Blood glucose (mg per 100 ml) | *Plasma* insulin (microunits per ml) | *Plasma growth* hormone (millimicro grams per ml) | Blood glucose (mg per 100 ml) | *Plasma* insulin (microunits per ml) | *Plasma growth* hormone (millimicro-grams per ml) |
| Fasting | 78 68 | 39 | 16 8 | 75 | 2 | 23 4 |
| 30 | 115 | 97 | | 136 | 2 | |
| 60 | | | | 124 | 6 | |
| 75 | 108 | 93 | | | | |
| 90 | | | | 167 | | |
| 120 | 106 | | 4 | 70 | | 9 |
| 180 | 70 | 70 | | 68 | <2 | |
| 240 | 17 | | 35 | 66 | | 28 |
| 300 | 24 | | 14 8 | 66 | | 16 4 |

Table 3 *Leucine tolerance test in Case 1*

0 15 g/kg orally

| Time (min) | Blood glucose (mg/100 ml) | Plasma insulin (microunits per ml) |
|---|---|---|
| Fasting | 82 | 6 |
| 0 | 67 | <5 |
| 40 | 62 | |
| 60 | 54 | |
| 90 | 60 | |
| 120 | 63 | |

therefore withdrawn At the request of the parents investigation was pursued no further

Tragically this child survived until the age of 6½ years She could swallow some liquid from a spoon but showed no other functions After the occurrence of hypoglycaemia in Case 2 she was investigated more fully in May 1968 Glucose loading was followed by hypoglycaemia in the 4 and 5 hour specimens (Table 2) There was no abnormal sensitivity to leucine (Table 3) and an attempt to induce ketosis was abandoned after 7 days on a diet with a fatty acid to glucose ratio of 3 1 The level of immunoreactive insulin in her serum was high in one fasting specimen (Table 2) but not in a second (Table 3) the subsequent rise in this level after glucose was not excessive but the level remained very high at 120 minutes Plasma growth hormone levels were suppressed and rose again normally after the glucose load (Table 2)

She died in October 1968 of bronchopneumonia Autopsy revealed gross cerebral cortical atrophy and microscopic neuronal loss in the parietal and occipital regions The loss was maximal in the outer zones of the cortex but in the watershed areas all neurones were lost The hippocampus, cerebellum and basal ganglia showed microscopic damage The distribution of the neuronal loss was not quite typical for hypoglycaemic damage does not usually involve the basal cortex and hippocampus There was slight hyperplasia of the pancreatic islets and the β-cells showed some nuclear polyploidy No adenomata were found

Fifth child (Case P R N) Male Group A Rhesus positive was born on April 5th 1966 after induction of labour at 34 weeks He was hydropic at birth and was not weighed Cord haemoglobin level was 9 g per 100 ml and a partial exchange transfusion was performed immediately followed by a normal exchange transfusion at 4 hours He progressed very well indeed after this and was never more than slightly jaundiced He weighed 1900 g at one week Physical and developmental progress were satisfactory At two months of age blood glucose estimations were performed after a 1 hour fast, and hourly for four hours after a milk formula The results were all in the range 60 80 mg per 100 ml At 14 months some habit movements of the face occurred for two or three weeks An E E G was performed to reassure the parents and was normal Development was assessed as that of a 12–14 month old normal child Normal development continued until he was two years old At this time his mother developed pulmonary tuberculosis and he had to be placed in a children's residential nursery He became rather withdrawn and frequently refused food Early one morning six weeks later he was found semi-conscious

When admitted to the Royal Children's Hospital three hours later he was still semi-conscious and was pale and sweating with a rapid weak pulse The clinical diagnosis of hypoglycaemia was soon confirmed by a Dextrostix reading of less than 40 mg per 100 ml a blood glucose estimation of 17 mg per 100 ml and a very gratifying response to intravenous glucose infusion He regained consciousness in 10–20 minutes and full normal alertness returned over 1–1½ hours When normal meals were started he was found to refuse everything other than milk and some carbohydrate foods Despite this he could sleep from 6 pm until 6 am without developing hypoglycaemia Dextrostix readings were always above 60 mg per 100 ml on awakening Consequently fasting from 10 pm to 10 am was considered safe as preparation for a glucose tolerance test On the morning of the test he awakened at 6 am as usual and seemed well However he became upset when other children in the ward ate breakfast and by 9 am he was rather quiet and drowsy At 9 15 am he convulsed and his blood glucose level was found to be 20 mg per 100 ml He responded quickly to intravenous glucose infusion No ketones were present in urine passed one hour later A glucose tolerance test was finally performed after six hours fasting and his blood glucose levels were normal up to five hours (Table 2) Glucagon 0 5 mg caused no rise in blood glucose from a level of 28 mg per 100 ml but 1 0 mg caused a reasonable response from a level of 81 mg per 100 ml After the first of these tests another hypoglycaemic attack occurred No attempt was made to give a ketogenic diet because it was considered to be impossible to persuade him to eat the diet A leucine tolerance test was not performed because his sister had shown no sensitivity and because his hypoglycaemic attacks had only occurred after fasting

Plasma insulin levels were low (Table 2) and plasma growth hormone responded normally to a glucose load despite a slightly elevated fasting level

Management has aimed at increasing his protein intake and avoiding prolonged fasting except when asleep Some carbohydrate food is given as soon as he awakens in the morning No further attacks have occurred in the last 5 months

## DISCUSSION

The two children described both suffered severe erythroblastosis foetalis and both devel

Table 1 *Blood sugar levels in Case 1 over first 2 days in hospital*

| | Day 1 | | Day 2 | |
|---|---|---|---|---|
| Time | Feeding[a] | Blood sugar[b] (mg per 100 ml) | Feeding[a] & Drugs | Blood sugar[b] (mg per 100 ml) |
| 8 00 a m | 180 ml | | | |
| 9 00 a m | | | | 52 |
| 10 00 a m | | 28 | 180 ml | 43 |
| 11 00 a m | | 56 | | 24 |
| 12 00 MD | 90 ml | 80 | | 55 |
| 1 00 p m | | 73 | I M Glucagon 0 2 mg | |
| 2 00 p m | 90 ml | 43 | 180 ml | 131 |
| 3 00 p m | | 27 | | 110 |
| 4 00 p m | | 46 | | 36 |

[a] Unmodified cow s milk
[b] Method of Aztoor & King (4)

ture labour at 35 weeks gestation Birth weight was 2070 g Jaundice developed 8 hours after birth and the direct Coombs test was positive Exchange transfusions were performed at 16 and 36 hours Subsequent progress has been normal

Third child Female group A Rhesus positive born August 8 1959 after induction of labour at 33 / weeks gestation Birth weight was 1530 g Cord haemoglobin level was 11 6 g per 100 ml Jaundice developed at 6 hours and exchange transfusions were performed at 14 40 and 90 hours of age She progressed very well until the 10th day when sudden loss of consciousness and circulatory collapse occurred Blood stained cerebrospinal fluid was obtained on lumbar puncture Blood sugar was not measured Death occurred on the 12th day

Necropsy revealed several areas of subarachnoid haemorrhage over the cerebellum which extended into the cortical tissue The pancreas was not examined microscopically

Fourth child (Case 1 M A N) Female Group A Rhesus positive born January 26 1962 after induction of labour at 35 weeks gestation Birth weight was 1200 g Cord blood haemoglobin level was 5[illegible] g per 100 ml Exchange transfusion was performed at 3 hours and jaundice was never severe (highest bilirubin level 19 9 mg per 100 ml on 9th day) so no further treatment was required Apnoeic attacks caused concern during the first 5 days of life but the blood sugar was not measured Solu Cortef had been given (10 mg every 6 hours) from birth because the platelet count was low Staphylococcal pneumonia developed and was very slow to clear She made good progress from the age of 3 months and 7 months and was then sitting unaided and developing normally One morning two weeks later she was found unconscious and convulsing She was admitted to another hospital but continued to convulse almost constantly for 3 /₂ weeks despite large doses of standard anticonvulsants and did not regain consciousness Lumbar puncture yielded normal cerebrospinal fluid and X rays of the skull were normal

On transfer to the Royal Children s Hospital on October 17 1962 she was unconscious with increased muscle tone and frequent minor twitchings of the limbs Blood sugar (total reducing substances) was measured 8 times daily for 2 days Figures below or near 30 mg per 100 ml were obtained 4 times and these all occurred 1–2 hours after a milk feeding (Table 1) Leucine sensitive hypoglycaemia was suspected but the child s condition prevented formal testing Frequent feeding with 10 dextrose and the use of I M Glucagon 0 15–0 2 mg 3 hourly were given in the hope of saving her life and cerebral function Some decrease in twitching and a gradual slight improvement in consciousness occurred over a week on this regime but it became clear that gross brain damage was present and that full recovery was not possible Pneumoencephalography later revealed gross generalised dilatation of the ventricular system with thinning of the cerebral cortex Active treatment was

Table 2 *Oral glucose tolerance test results*

| | Case 1 (1 6 g/kg) | | | Case 2 (2 2 g/kg) | | |
|---|---|---|---|---|---|---|
| Time (min) | Blood glucose (mg per 100 ml) | Plasma insulin (microunits per ml) | Plasma growth hormone (millimicro grams per ml) | Blood glucose (mg per 100 ml) | Plasma insulin (microunits per ml) | Plasma growth hormone (millimicro grams per ml) |
| Fasting | 78 68 | 39 | 16 8 | 75 | <2 | 23 4 |
| 30 | 115 | 97 | | 136 | 4 | |
| 60 | | | | 124 | 6 | |
| 75 | 108 | 93 | | | | |
| 90 | | | | 167 | | |
| 120 | 106 | | 4 | 70 | | 9 |
| 180 | 70 | 70 | | 68 | <2 | |
| 240 | 17 | | 35 | 66 | | 28 |
| 300 | 24 | | 14 8 | 66 | | 16 4 |

Table 3 *Leucine tolerance test in Case 1*

0 15 g/kg orally

| Time (min) | Blood glucose (mg/100 ml) | Plasma insulin (microunits per ml) |
|---|---|---|
| Fasting | 82 | 6 |
| 20 | 67 | <5 |
| 40 | 62 | |
| 60 | 54 | |
| 90 | 60 | |
| 120 | 63 | |

therefore withdrawn At the request of the parents investigation was pursued no further

Tragically this child survived until the age of 6 1/2 years She could swallow some liquid from a spoon but showed no other functions After the occurrence of hypoglycaemia in Case 2 she was investigated more fully in May 1968 Glucose loading was followed by hypoglycaemia in the 4 and 5 hour specimens (Table 2) There was no abnormal sensitivity to leucine (Table 3) and an attempt to induce ketosis was abandoned after 7 days on a diet with a fatty acid to glucose ratio of 3 1 The level of immunoreactive insulin in her serum was high in one fasting specimen (Table 2) but not in a second (Table 3) the subsequent rise in this level after glucose was not excessive but the level remained very high at 180 minutes Plasma growth hormone levels were suppressed and rose again normally after the glucose load (Table 2)

She died in October 1968 of bronchopneumonia Autopsy revealed gross cerebral cortical atrophy and microscopic neuronal loss in the parietal and occipital regions The loss was maximal in the outer zones of the cortex but in the "watershed areas" all neurones were lost The hippocampus cerebellum and basal ganglia showed microscopic damage The distribution of the neuronal loss was not quite typical for hypoglycaemic damage does not usually involve the visual cortex and hippocampus There was slight hyperplasia of the pancreatic islets and the β-cells showed some nuclear polyploidy No adenomata were found

Fifth child (Case 3 P R N ) Male Group A Rhesus positive was born on April 5th 1966 after induction of labour at 34 weeks He was hydropic at birth and was not weighed Cord haemoglobin level was 9 g per 100 ml and a partial exchange transfusion was performed immediately followed by a normal exchange transfusion at 24 hours He progressed very well indeed after this and was never more than slightly jaundiced He weighed 1900 g at one week Physical and developmental progress were satisfactory At two months of age blood glucose estimations were performed after a 12 hour fast and hourly for four hours after a milk formula The results were all in the range 60 80 mg per 100 ml At 14 months some habit movements of the face occurred for two or three weeks An E.E.G was performed to reassure the parents and was normal Development was assessed as that of a 12 14 month old normal child Normal development continued until he was two years old At this time his mother developed pulmonary tuberculosis and he had to be placed in a children's residential nursery He became rather withdrawn and frequently refused food Early one morning six weeks later he was found semi-conscious

When admitted to the Royal Children's Hospital three hours later he was still semi-conscious and was pale and sweating with a rapid weak pulse The clinical diagnosis of hypoglycaemia was soon confirmed by a Dextrostix reading of less than 40 mg per 100 ml a blood glucose estimation of 17 mg per 100 ml and a very gratifying response to intravenous glucose infusion He regained consciousness in 10-20 minutes and full normal alertness returned over 8-12 hours When normal meals were started he was found to refuse everything other than milk and some carbohydrate foods Despite this he could sleep from 6 p m until 6 a m without developing hypoglycaemia Dextrostix readings were always above 60 mg per 100 ml on awakening Consequently fasting from 10 p m to 10 a m was considered safe as preparation for a glucose tolerance test On the morning of the test he awakened at 6 a.m. as usual and seemed well However he became upset when other children in the ward ate breakfast, and by 9 a.m he was rather quiet and drowsy At 9 15 a m he convulsed and his blood glucose level was found to be 20 mg per 100 ml He responded quickly to intravenous glucose infusion No ketones were present in urine passed one hour later A glucose tolerance test was finally performed after six hours fasting and his blood glucose levels were normal up to five hours (Table 2) Glucagon 0 5 mg caused no rise in blood glucose from a level of 28 mg per 100 ml but 1 0 mg caused a reasonable response from a level of 81 mg per 100 ml After the first of these tests another hypoglycaemic attack occurred No attempt was made to give a ketogenic diet because it was considered to be impossible to persuade him to eat the diet A leucine tolerance test was not performed because his sister had shown no sensitivity and because his hypoglycaemic attacks had only occurred after fasting

Plasma insulin levels were low (Table 2) and plasma growth hormone responded normally to a glucose load despite a slightly elevated fasting level

Management has aimed at increasing his protein intake and avoiding prolonged fasting except when asleep Some carbohydrate food is given as soon as he awakens in the morning No further attacks have occurred in the last 5 months.

## DISCUSSION

The two children described both suffered severe erythroblastosis foetalis and both devel

Table 1 *Blood sugar levels in Case 1 over first 2 days in hospital*

| | Day 1 | | Day 2 | |
|---|---|---|---|---|
| Time | Feeding[a] | Blood sugar[b] (mg per 100 ml) | Feeding[a] & Drugs | Blood sugar[b] (mg per 100 ml) |
| 8 00 a m | 180 ml | | | |
| 9 00 a m | | | | 52 |
| 10 00 a m | | 28 | 180 ml | 43 |
| 11 00 a m | | 56 | | 24 |
| 12 00 MD | 90 ml | 80 | | 55 |
| 1 00 p m | | 73 | I M Glucagon 0 2 mg | |
| 2 00 p m | 90 ml | 43 | 180 ml | 131 |
| 3 00 p m | | 27 | | 110 |
| 4 00 p m | | 46 | | 36 |

[a] Unmodified cow s milk
[b] Method of Aratoor & King (4)

ture labour at 35 weeks gestation Birth weight was 2070 g Jaundice developed 8 hours after birth and the direct Coombs test was positive Exchange transfusions were performed at 16 and 36 hours Subsequent progress has been normal

Third child Female group A Rhesus positive born August 8 1959 after induction of labour at 33 /₂ weeks gestation Birth weight was 1530 g Cord haemoglobin level was 11 6 g per 100 ml Jaundice developed at 6 hours and exchange transfusions were performed at 14 40 and 90 hours of age She progressed very well until the 10th day when sudden loss of consciousness and circulatory collapse occurred Blood stained cerebrospinal fluid was obtained on lumbar puncture Blood sugar was not measured Death occurred on the 12th day

Necropsy revealed several areas of subarachnoid haemorrhage over the cerebellum which extended into the cortical tissue The pancreas was not examined microscopically

Fourth child (Case 1 M A N) Female Group A Rhesus positive born January 26 1962 after induction of labour at 35 weeks gestation Birth weight was 1200 g Cord blood haemoglobin level was 51 g per 100 ml Exchange transfusion was performed at 3 hours and jaundice was never severe (highest bilirubin level 19 9 mg per 100 ml on 9th day) so no further treatment was required Apnoeic attacks caused concern during the first 5 days of life but the blood sugar was not measured Solu Cortef had been given (10 mg every 6 hours) from birth because the platelet count was low Staphylococcal pneumonia developed and was very slow to clear She made good progress from the age of 3 months and 7 months and was then sitting unaided and developing normally One morning two weeks later she was found unconscious and convulsing She was admitted to another hospital but continued to convulse almost constantly for 3 / weeks despite large doses of standard anticonvulsants and did not regain consciousness Lumbar puncture yielded normal cerebrospinal fluid and X rays of the skull were normal

On transfer to the Royal Children s Hospital on October 12 1962 she was unconscious with increased muscle tone and frequent minor twitchings of the limbs Blood sugar (total reducing substances) was measured 8 times daily for 2 days Figures below or near 30 mg per 100 ml were obtained 4 times and these all occurred 1 2 hours after a milk feeding (Table 1) Leucine sensitive hypoglycaemia was suspected but the child s condition prevented formal testing Frequent feeding with 10 dextrose and the use of I M Glucagon 0 15–0 2 mg 3 hourly were given in the hope of saving her life and cerebral function Some decrease in twitching and a gradual slight improvement in consciousness occurred over a week on this regime but it became clear that gross brain damage was present and that full recovery was impossible Pneumoencephalography later revealed gross generalised dilatation of the ventricular system with thinning of the cerebral cortex Active treatment was

Table 2 *Oral glucose tolerance test results*

| | Case 1 (1 6 g/kg) | | | Case 2 (2 2 g/kg) | | |
|---|---|---|---|---|---|---|
| Time (min) | Blood glucose (mg per 100 ml) | Plasma insulin (microunits per ml) | Plasma growth hormone (millimicro grams per ml) | Blood glucose (mg per 100 ml) | Plasma insulin (microunits per ml) | Plasma growth hormone (millimicro grams per ml) |
| Fasting | 78 68 | 39 | 16 8 | 75 | 2 | 23 4 |
| 30 | 115 | 97 | | 136 | 2 | |
| 60 | | | | 124 | 6 | |
| 75 | 108 | 93 | | | | |
| 90 | | | | 167 | | |
| 120 | 106 | | 4 | 70 | | 9 |
| 180 | 70 | 70 | | 68 | 2 | |
| 240 | 17 | | 35 | 66 | | 23 |
| 300 | 24 | | 14 8 | 66 | | 16 4 |

Table 3 *Leucine tolerance test in Case 1*

0.15 g/kg orally

| Time (min) | Blood glucose (mg/100 ml) | Plasma insulin (microunits per ml) |
|---|---|---|
| Fasting | 82 | 6 |
| 0 | 67 | <5 |
| 40 | 62 | |
| 60 | 54 | |
| 90 | 60 | |
| 120 | 63 | |

therefore withdrawn. At the request of the parents investigation was pursued no further.

Tragically this child survived until the age of 6 / years. She could swallow some liquid from a spoon but showed no other functions. After the occurrence of hypoglycaemia in Case 2 she was investigated more fully in May 1968. Glucose loading was followed by hypoglycaemia in the 4 and 5 hour specimens (Table 2). There was no abnormal sensitivity to leucine (Table 3) and an attempt to induce ketosis was abandoned after 7 days on a diet with a fatty acid to glucose ratio of 3 1. The level of immunoreactive insulin in her serum was high in one fasting specimen (Table 2) but not in a second (Table 3); the subsequent rise in this level after glucose was not excessive but the level remained very high at 1 0 minutes. Plasma growth hormone levels were suppressed and rose again normally after the glucose load (Table ?).

She died in October 1968 of bronchopneumonia. Autopsy revealed gross cerebral cortical atrophy and microscopic neuronal loss in the parietal and occipital regions. The loss was maximal in the outer zones of the cortex but in the watershed areas all neurones were lost. The hippocampus cerebellum and basal ganglia showed microscopic damage. The distribution of the neuronal loss was not quite typical for hypoglycaemic damage does not usually involve the visual cortex and hippocampus. There was slight hyperplasia of the pancreatic islets and the β-cells showed some nuclear polyploidy. No adenomata were found.

Fifth child (Case 2. P. R. N.) Male. Group A Rhesus positive was born on April 5th 1966 after induction of labour at 34 weeks. He was hydropic at birth and was not weighed. Cord haemoglobin level was 9 g per 100 ml and a partial exchange transfusion was performed immediately followed by a normal exchange transfusion at 24 hours. He progressed very well indeed after this and was never more than slightly jaundiced. He weighed 1900 g at one week. Physical and developmental progress were satisfactory. At two months of age blood glucose estimations were performed after a 3 hour fast and hourly for four hours after a milk formula. The results were all in the range 60-80 mg per 100 ml. At 14 months some tonic movements of the face occurred for two or three weeks. An E.E.G. was performed to reassure the parents and was normal. Development was assessed as that of a 12–14 month old normal child. Normal development continued until he was two years old. At this time his mother developed pulmonary tuberculosis and he had to be placed in a children's residential nursery. He became rather withdrawn and frequently refused food. Early one morning six weeks later he was found semi-conscious.

When admitted to the Royal Children's Hospital three hours later he was still semi-conscious and was pale and sweating with a rapid weak pulse. The clinical diagnosis of hypoglycaemia was soon confirmed by a Dextrostix reading of less than 40 mg per 100 ml, a blood glucose estimation of 17 mg per 100 ml and a very gratifying response to intravenous glucose infusion. He regained consciousness in 10–30 minutes and full normal alertness returned over 8–12 hours. When normal meals were started he was found to refuse everything other than milk and some carbohydrate foods. Despite this he could sleep from 6 p.m. until 6 a.m. without developing hypoglycaemia. Dextrostix readings were always above 60 mg per 100 ml on awakening. Consequently fasting from 10 p.m. to 10 a.m. was considered safe in preparation for a glucose tolerance test. On the morning of the test he awakened at 6 a.m. as usual and seemed well. However he became upset when other children in the ward ate breakfast and by 9 a.m. he was rather quiet and drowsy. At 9.15 a.m. he convulsed and his blood glucose level was found to be 20 mg per 100 ml. He responded quickly to intravenous glucose infusion. No ketones were present in urine passed one hour later. A glucose tolerance test was finally performed after six hours fasting and his blood glucose levels were normal up to five hours (Table 2). Glucagon 0.5 mg caused no rise in blood glucose from a level of 28 mg per 100 ml but 1.0 mg caused a reasonable response from a level of 31 mg per 100 ml. After the first of these tests another hypoglycaemic attack occurred. No attempt was made to give a ketogenic diet because it was considered to be impossible to persuade him to eat the diet. A leucine tolerance test was not performed because his sister had shown no sensitivity and because his hypoglycaemic attacks had only occurred after fasting.

Plasma insulin levels were low (Table 2) and plasma growth hormone responded normally to a glucose load despite a slightly elevated fasting level.

Management has aimed at increasing his protein intake and avoiding prolonged fasting except when asleep. Some carbohydrate food is given as soon as he awakens in the morning. No further attacks have occurred in the last 5 months.

## DISCUSSION

The two children described both suffered severe erythroblastosis foetalis and both devel

Table 1 *Blood sugar levels in Case 1 over first 2 days in hospital*

| Time | Day 1 Feeding | Day 1 Blood sugar[b] (mg per 100 ml) | Day 2 Feeding[a] & Drugs | Day 2 Blood sugar[b] (mg per 100 ml) |
|---|---|---|---|---|
| 8 00 a m | 180 ml | | | |
| 9 00 a m | | | | 52 |
| 10 00 a m | | 28 | 180 ml | 43 |
| 11 00 a m | | 56 | | 24 |
| 12 00 MD | 90 ml | 80 | | 55 |
| 1 00 p m | | 73 | I M Glucagon 0 2 mg | |
| 2 00 p m | 90 ml | 43 | 180 ml | 131 |
| 3 00 p m | | 27 | | 110 |
| 4 00 p m | | 46 | | 36 |

[a] Unmodified cow s milk
[b] Method of Asatoor & King (4)

ture labour at 35 weeks gestation Birth weight was 2070 g Jaundice developed 8 hours after birth and the direct Coombs test was positive Exchange transfusions were performed at 16 and 36 hours Subsequent progress has been normal

Third child Female group A Rhesus positive born August 8 1959 after induction of labour at 33 / weeks gestation Birth weight was 1530 g Cord haemoglobin level was 11 6 g per 100 ml Jaundice developed at 6 hours and exchange transfusions were performed at 14 40 and 90 hours of age She progressed very well until the 10th day when sudden loss of consciousness and circulatory collapse occurred Blood stained cerebrospinal fluid was obtained on lumbar puncture Blood sugar was not measured Death occurred on the 12th day

Necropsy revealed several areas of subarachnoid haemorrhage over the cerebellum which extended into the cortical tissue The pancreas was not examined microscopically

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On transfer to the Royal Childrens Hospital on October 12 1962 she was unconscious with increased muscle tone and frequent minor twitchings of the limbs Blood sugar (total reducing substances) was measured 8 times daily for 2 days Figures below or near 30 mg per 100 ml were obtained 4 times and these all occurred 1–2 hours after a milk feeding (Table 1) Leucine sensitive hypoglycaemia was suspected but the child s condition prevented formal testing Frequent feeding with 10 dextrose and the use of I M Glucagon 0 15–0 2 mg 3 hourly were given in the hope of saving her life and cerebral function Some decrease in twitching and a gradual slight improvement in consciousness occurred over a week on this regime but it became clear that gross brain damage was present and that full recovery was impossible Pneumoencephalography later revealed gross generalized dilatation of the ventricular system with thinning of the cerebral cortex Active treatment was

Table 2 *Oral glucose tolerance test results*

| Time (min) | Case 1 (1 6 g/kg) Blood glucose (mg per 100 ml) | Case 1 Plasma insulin (microunits per ml) | Case 1 Plasma growth hormone (millimicrograms per ml) | Case 2 (2 2 g/kg) Blood glucose (mg per 100 ml) | Case 2 Plasma insulin (microunits per ml) | Case 2 Plasma growth hormone (millimicrograms per ml) |
|---|---|---|---|---|---|---|
| Fasting | 78 68 | 39 | 16 8 | 75 | <2 | 23 4 |
| 30 | 115 | 97 | | 136 | 2 | |
| 60 | | | | 1 4 | 6 | |
| 75 | 108 | 93 | | | | |
| 90 | | | | 167 | | |
| 120 | 106 | | 4 | 70 | | 9 |
| 180 | 70 | 70 | | 68 | <2 | |
| 240 | 17 | | 35 | 66 | | 28 |
| 300 | <4 | | 14 8 | 66 | | 16 4 |

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*Key words:* Hypoglycaemia, erythroblastosis foetalis, islet cell hyperplasia

oped hypoglycaemia after the newborn period It seems possible that hypoglycaemia may have caused the apnoeic attacks described in Case 1 in the first week of life

For the reasons outlined in the introductory comments it seems reasonable to propose that the hypoglycaemia was a late sequel of erythroblastosis foetalis It is suggested that the disturbance of carbohydrate metabolism which causes islet cell hyperplasia in the erythroblastic foetus can persist for some years The analogous situation in babies who are small for dates provides a precedent for this suggestion Our limited studies do not provide any basis for speculation regarding the way in which erythroblastosis interferes with carbohydrate metabolism The cases are reported to stimulate research into this topic and to suggest to clinicians that they should add erythroblastosis to the list of disturbances during pregnancy which should make them think of hypoglycaemia as the cause of convulsions in a child The plasma insulin levels are very different in the two children and no adequate explanation is obvious The low levels found in Case 2 despite an abnormally prolonged hyperglycaemic phase could be compatible with an abnormal sensitivity to insulin An increased sensitivity to the effect of insulin plus an inefficient insulin response to hyperglycaemia might explain the rise fall rise sequence in the glucose tolerance test of Case 2 This is not thought to be a technical error for we have seen a similar pattern in other cases of childhood hypoglycaemia, and others have commented on this feature (8) It seems as though the feed back control of the glucose levels are very crude and allow overshooting in both directions

Another observation of interest and of some practical assistance relates to the affect the sight of food has upon blood glucose Case 2 is able to sleep 12 hours every night and his blood glucose is always above 60 mg per 100 ml on waking However he twice became hypoglycaemic with shorter fasts, when he was awake for the last 3 hours and watched others eat during this time This phenomenon is of theoretical interest and may support the existence of an upper gastrointestinal stimulus of insulin secretion In practice this effect may be useful when trying to reveal a hypoglycaemic tendency, and must be remembered when planning the safe length of a fast before tolerance tests in patients who are known to develop fasting hypoglycaemia

## SUMMARY

Hypoglycaemia is described in two siblings at seven months and 25 months of age respectively Both had suffered very severe erythroblastosis foetalis and it is suggested that the hypoglycaemia represents a late sequel of this disease Erythroblastosis should be added to the list of conditions associated with hypoglycaemia in the newborn and also in older children During the investigations an influence of the sight of food upon blood glucose levels was noted

## ACKNOWLEDGEMENT

I am indebted to Dr J F Connelly for glucose estimations and Dr F I R Martin for insulin and growth hormone assays

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Table I. Major clinical and laboratory findings at the time of biopsy and results of histologic and immunofluorescent studies

| Case | Sex | Age at onset | Age at biopsy y | Urinary protein g/L | Urinary protein g/24 h | Haematuria | CH50 | Complicated | Histopathology of glomeruli: Epith cell pr | Endocap cell pr | BM | MES | Immunofluorescence of glomeruli: IgG | IgA | IgM | C' | Fibrin | Albumin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolated proteinuria | | | | | | | | | | | | | | | | | | |
| 1 | ♂ | 1 6/12 | 1 12 | | | | 105 | | | + | | | | | | ± | ± | − |
| 2 | | 9 | 9 8/12 | 4 | | | 30 | | | | | | | | + | + | − | − |
| 3 | | | 6 12 | 0.3 | | | | | | + | | + | | | + | ++ | − | − |
| 4 | ♂ | 7 71 | 9 71 | 0.3 | | | 151 | | | | | | | | + | ++ | + | − |
| Nephrotic syndrome | | | | | | | | | | | | | | | | | | |
| 5 | | 11 6/1 | 11 11/12 | | 5 | | 114 | | | | − | ++ | | | + | ++ | − | − |
| 6 | | 2 6/12 | 3 /12 | 0.5 | | | | | | | + | | | | ++ | ++ | − | − |
| 7 | ♂ | 4 6/12 | 4 10/12 | 3 | | | | | + | + | − | − | | | + | +++ | ± | − |
| 8 | ♂ | 2 6/12 | 15 /12 | 0.5 | | | 140 | | | + | ± | ± | ± | | | ++ | ± | − |
| 9 | ♂ | 3 10/12 | 5 | | | | | | | | + | + | | | ++ | +++ | ± | − |
| 10 | | 13 10/1 | 14 | | 0.5 | | 118 | | | + | | + | + | + | ++ | ++ | + | − |
| Proteinuria and haematuria | | | | | | | | | | | | | | | | | | |
| 11 | ♂ | 6 5/12 | 8 | | 2.5 | | 136 | | + | + | | ± | ± | | − | ± | − | − |
| 12 | ♀ | 4 11/12 | 5 /12 | 1 | | + | 100 | | | | ± | | | | ± | + | ± | − |
| 13 | ♂ | 5 7/12 | 7 1/12 | | 1.5 | | 110 | | + | + | ± | + | − | | ± | ± | + | − |
| 14 | | 9 9/12 | 10 8/12 | | 2 | + | 118 | | + | + | + | ++ | | | + | + | ± | − |
| 15 | ♂ | 6 2/1 | 10 | 0.3 | | | 56 | | + | + | ++ | ++ | + | − | + | +++ | ± | − |
| 16 | ♂ | 7 2/12 | 7 10/12 | | 1.3 | + | 150 | | + | + | + | + | + | ++ | ++ | ++ | +++ | − |
| 17 | ♂ | 4 7/12 | 8 7/12 | 1 | | + | 90 | | | | | | ++ | ++ | +++ | +++ | +++ | − |
| 18 | ♀ | 5 | 6 6/12 | 0.3 | | + | 160 | | ± | ± | + | + | + | ++ | + | ++ | +++ | + |
| 19 | | 8 2/12 | 9 4/12 | 6 | | + | 106 | | ++ | ++ | ++ | ++ | +++ | +++ | ++ | +++ | +++ | ± |
| Nephrotic syndrome and haematuria | | | | | | | | | | | | | | | | | | |
| 0 | | 8 7/12 | 8 11/12 | 3 | | + | 178 | | + | + | ± | + | | − | ± | + | ± | − |
| 21 | ♀ | 14 1/1 | 14 4/12 | | 4 | + | 4 | | ± | ++ | ++ | ++ | + | | + | +++ | + | − |
| 22 | ♀ | 4 10/12 | 6 1/12 | 0.3 | | + | | − | + | ++ | ++ | ++ | +++ | − | + | +++ | + | − |
| 23 | ♂ | 7 | 7 4/12 | 6 | | + | 112 | − | + | ++ | ++ | ++ | +++ | +++ | +++ | +++ | +++ | − |
| Anaphylactoid purpura | | | | | | | | | | | | | | | | | | |
| 24 | ♂ | 3 8/12 | 3 11/12 | 2 | | + | 166 | − | ± | + | ± | + | ± | ++ | ++ | ++ | +++ | ± |
| 25 | ♂ | 7 11/12 | 8 9/12 | 1.5 | | + | 81 | − | − | ++ | ± | + | ± | ++ | ++ | ++ | +++ | ++ |
| Hereditary diffuse nephropathy | | | | | | | | | | | | | | | | | | |
| 26 | ♂ | 10 7/12 | 12 | — | | + | 91 | | − | ++ | ± | + | − | − | ± | + | ± | − |
| 27 | ♂ | 2 | 8 | 0.8 | | + | | − | − | ++ | ± | + | − | − | ± | ++ | + | − |
| 28 | ♀ | 6 _/12 | 15 2/12 | 0.3 | | + | | − | − | ± | ± | ± | − | − | + | ++ | + | − |
| 29 | ♂ | 4 8/12 | 8 8/12 | 0.3 | | + | 95 | − | − | + | ± | ± | − | − | ± | + | + | − |
| 30 | ♂ | 1? 11/12 | 14 1/12 | | 9 | + | 100 | + | | + | + | ++ | − | − | ± | +++ | ++ | − |

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The presence of immunoglobulin deposits in kidneys from patients with diffuse nephropathies was first described by Mellors and Ortega (19) These studies were extended (3 7 8 9 13 14 15 16 24 26) by the determination of the type of immunoglobulin present in the diseased glomeruli and by the demonstration of complement and fibrin or fibrinogen In these investigations the direct immunofluorescent technique was used as first described by Coons (1)

The main purpose of this paper is to give the results obtained with an indirect immunofluorescent technique for the demonstration of IgG IgA IgM complement fibrinogen and albumin in glomeruli of children with diffuse nephropathies Sections used in the immunofluorescent technique were restained for light microscopy and the localization of the protein deposits with regard to glomerular structure was facilitated by comparison of fluorescent and light microscopic pictures Correlations were made between the immunofluorescent light microscopic and clinical manifestations

An attempt was made to demonstrate M proteins of streptococci in the renal biopsy specimens In addition sera from patients with diffuse nephropathies were studied for the presence of antibodies to normal kidney tissue

## MATERIALS AND METHODS

*Clinical definitions*

Haematuria is defined as the excretion of more than 30 000 erythrocytes per minute as determined by Addis count

Proteinuria was estimated by the Biuret method and expressed as grams per litre of urine or as grams excreted per 24 hours Table I shows the amount of proteinuria at the time of renal biopsy The term complicated is used whenever proteinuria and haematuria were accompanied by hypertension or chronic impairment of renal function

The term nephrotic syndrome is used whenever in the course of any disease the patient demonstrated proteinuria accompanied by generalized oedema or a plasma albumin level of less than [illegible] gram per litre

*Patients*

Patients were classified on basis of clinical symptomatology (Table 1)

1 Isolated proteinuria (case 1–4) Examination of the overnight urine specimens excluded postural proteinuria
2 Nephrotic syndrome (case 5–10) Haematuria was absent in these children
3 Proteinuria and haematuria without nephrotic syndrome (case 11–19)
4 Nephrotic syndrome with haematuria (case 20–23)
5 Anaphylactoid Purpura (case 24 25) Both patients showed proteinuria and haematuria
6 Hereditary diffuse nephropathy (case 26–30) Cases 26 27 and 28 were related to each other Inner ear deafness was found in two patients (case 29 and case 30) both showed a nephrotic syndrome with haematuria

Table 1 Major clinical and laboratory findings at the time of biopsy and results of histologic and immunofluorescent studies

| Case | Sex | Age at onset | Age at biopsy | Urinary protein g/L | Urinary protein g/24 h | Haematuria | CH50 | Complicated | Histopathology of glomeruli: Epith cell pr | Endoc p cell pr | BM | MES | Immunofluorescence of glomeruli: IgG | IgA | IgM | C' | Fibrin | Albumin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolated proteinuria | | | | | | | | | | | | | | | | | | |
| 1 | ♂ | 1 6/12 | 2 1/12 | | 2 | − | 105 | | + | ± | − | + | | − | − | ± | ± | − |
| 2 | ♀ | 9 | 9 8/12 | 4 | | − | 90 | − | | | | | | − | ± | + | − | − |
| 3 | ♀ | | 6 2/12 | 0.5 | | − | | − | + | ± | − | ± | | − | + | ++ | − | − |
| 4 | ♂ | 7 7/1 | 9 7/12 | 0.3 | | | 151 | | ± | + | | + | + | − | + | ++ | + | − |
| Nephrotic syndrome | | | | | | | | | | | | | | | | | | |
| 5 | ♀ | 11 6/12 | 11 11/12 | | 5 | | 114 | − | ± | + | − | ++ | | − | + | ++ | − | − |
| 6 | ♀ | 2 6/1 | 3 2/12 | 0.5 | | | | − | | + | ± | + | | − | ++ | ++ | − | − |
| 7 | ♂ | 4 8/12 | 4 10/12 | 3 | | | | | ± | + | − | ± | | − | + | +++ | ± | − |
| 8 | ♂ | 2 6/1 | 15 2/12 | 0.5 | | | 140 | | | + | ± | ± | ± | | + | ++ | ± | − |
| 9 | ♂ | 3 10/1 | 5 | | 2 | | | | ± | | + | + | | | ++ | +++ | ± | − |
| 10 | ♀ | 13 10/12 | 14 | | 0.5 | | 118 | | | + | − | + | + | + | ++ | ++ | + | − |
| Proteinuria and haematuria | | | | | | | | | | | | | | | | | | |
| 11 | ♂ | 6 3/1 | 8 | | 2.5 | + | 136 | | ± | ± | − | ± | ± | − | − | ± | − | − |
| 12 | ♂ | 4 11/1? | 5 2/12 | 1 | | + | 100 | | | | ± | + | − | | ± | + | ± | − |
| 13 | ♂ | 5 7/12 | 7 1/1 | | 1.5 | + | 110 | | ± | + | ± | + | − | − | ± | ± | + | − |
| 14 | ♀ | 9 9/12 | 10 8/12 | | 2 | + | 118 | | ± | + | + | ++ | − | − | + | + | ± | − |
| 15 | ♂ | 6 2/1 | 10 | 0.3 | | + | 56 | + | ++ | ++ | ++ | ++ | + | | + | +++ | ± | − |
| 16 | ♂ | 7 /1 | 7 10/12 | | 1.3 | + | 190 | | ± | + | ± | + | + | ++ | ++ | ++ | +++ | − |
| 17 | ♂ | 4 7/12 | 8 7/12 | 1 | | + | 90 | | | | | | ++ | ++ | +++ | +++ | +++ | − |
| 18 | ♀ | 5 | 6 6/12 | 0.3 | | + | 160 | | ± | ± | ± | + | + | ++ | + | ++ | +++ | + |
| 19 | ♀ | 8 2/12 | 9 4/12 | 2.6 | | + | 106 | | ++ | ++ | ++ | ++ | +++ | +++ | ++ | +++ | +++ | ± |
| Nephrotic syndrome and haematuria | | | | | | | | | | | | | | | | | | |
| 20 | ♀ | 8 7/12 | 8 11/12 | 3 | | + | 1.8 | | + | + | ± | + | − | − | ± | + | ± | − |
| 21 | ♀ | 14 1/12 | 14 4/1 | | 4 | + | 4 | | ± | ++ | ++ | ++ | + | − | + | +++ | + | − |
| 22 | ♀ | 4 10/12 | 6 1/12 | 0.3 | | + | | | + | ++ | ++ | ++ | +++ | − | + | +++ | + | − |
| 23 | ♂ | 7 | 7 4/12 | 6 | | + | 112 | | ± | ++ | ++ | ++ | +++ | +++ | +++ | +++ | +++ | − |
| Anaphylactoid purpura | | | | | | | | | | | | | | | | | | |
| 24 | ♂ | 3 9/12 | 3 11/12 | 2 | | + | 166 | − | ± | + | ± | + | ± | ++ | ++ | ++ | +++ | ± |
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| 27 | ♂ | 2 | 8 | 0.8 | | + | | − | − | ++ | ± | + | − | − | ± | ++ | + | − |
| 28 | ♀ | 6 7/12 | 15 2/12 | 0.3 | | + | | − | − | + | ± | ± | − | − | + | ++ | + | − |
| 29 | ♂ | 4 8/12 | 8 8/12 | 0.3 | | + | 95 | − | − | + | ± | ± | − | − | ± | + | + | − |
| 30 | ♀ | 12 11/12 | 14 1/12 | | 9 | + | 100 | + | − | + | + | ++ | − | − | + | +++ | ++ | − |

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The main purpose of this paper is to give the results obtained with an indirect immunofluorescent technique for the demonstration of IgG IgA IgM complement fibrinogen and albumin in glomeruli of children with diffuse nephropathies Sections used in the immunofluorescent technique were restained for light microscopy and the localization of the protein deposits with regard to glomerular structure was facilitated by comparison of fluorescent and light microscopic pictures Correlations were made between the immunofluorescent light microscopic and clinical manifestations

An attempt was made to demonstrate M proteins of streptococci in the renal biopsy specimens In addition sera from patients with diffuse nephropathies were studied for the presence of antibodies to normal kidney tissue

## MATERIALS AND METHODS

### *Clinical definitions*

Haematuria is defined as the excretion of more than 30 000 erythrocytes per minute as determined by Addis count

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The term *nephrotic syndrome* is used whenever in the course of any disease the patient demonstrated proteinuria accompanied by generalized oedema or a plasma albumin level of less than 25 gram per litre

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5. Anaphylactoid Purpura (case 24 25) Both patients showed proteinuria and haematuria
6. Hereditary diffuse nephropathy (case 26–30) Cases 26 27 and 28 were related to each other Inner ear deafness was found in two patients (case 29 and case 30) both showed a nephrotic syn

scope equipped with a Philips CS 150 W mercury light source appropriate filters (UG K 430) and a dark field condensor

As control for the specificity of the observed immunofluorescent staining, a negative result was required when sections were flooded with normal rabbit serum instead of with the antiserum Fluorescence was subjectively graded (– to + + +) depending on the extent

Photographs were taken with a Leitz Orthomat camera using Ansco 200 for the fluorescent sections and Ektachrome Reversal Print for the P A S M / H E stained sections

The indirect immunofluorescent technique was used for the detection of kidney antibodies in the sera of our patients (This investigation was not done in cases 2 3 1 27 28 Table 1) Cryostat sections of rat kidney (unfixed) and human kidney (unfixed and fixed in acetone or ethanol 96 or formalin/macrodex) in which no immunoglobulin fixation could be demonstrated were incubated with the patients serum (undiluted and diluted /₈ with buffered saline pH 7 ) Eventual immunoglobulin fixation could then be visualized by the fluorescent horse anti human immuno lobulin reagent Looking for complement binding anti kidney antibodies normal human kidney sections were flooded with fresh human AB serum after incubation of the sections with the patient's serum As third layer the fluorescent rabbit anti human complement reagent was employed All incubations were done at room temperature (6)

## RESULTS AND DISCUSSION

In the past the direct technique has always been used in immunofluorescence studies of the kidney The use of the indirect immunofluorescent technique however has certain advantages Only a single serum must be labelled with F I T C This serum contains antibodies *against* the IgG fraction of the antisera applied to the renal cryostat sections as first layer all of which originate from the same animal species Moreover the indirect flourescent technique affords a possibility of simple and reliable verification of the specificity of the fluorescence observed If normal rabbit serum instead of rabbit antiserum is used a first layer then no fluorescence should occur Finally the fact that only one flurescent conjugate is used enhances the comparability of results obtained with the various antisera

When protein precipitates have been established in renal sections their localization can be studied by re staining the same section with P A S M /H E The re staining technique used in this study has the great advantage that precipitates can be located by light microscopy in the same section in which they were demonstrated by the fluorescent technique This particularly facilitates the localization of precipitates in relation to the basement membrane It is necessary for adequate assessment to have sections not thicker than 2 $\mu$ This causes some technical difficulties when the renal tissue has been obtained by needle biopsy

In 8 of the 11 *control patients* IgM and complement were observed in the mesangium In addition extensive protein precipitates were present in degenerating sclerosing glomeruli despite the fact that none of the control patients showed any histological sign of glomerulonephritis Freedman *et al* (8) found traces of $\gamma$ globulin in the mesangium of their 9 control patients Kobayashi (13) found no immunoglobulins at all in 14 controls but unlike ourselves Kobayashi used post mortem material Okuda *et al* (22) found $\gamma$ globulin and complement in the mesangium in experimental aminonucleoside nephritis although probably no immunological mechanisms are involved in this It therefore seems possible that complement and immunoglobulins may be observed in the mesangium of glomeruli without a coexisting immunopathy

These facts should be born in mind when interpreting the findings obtained in the 10 children with *isolated proteinuria or an uncomplicated nephrotic syndrome* (Table 1) In these children the mesangium (notably the region of the afferent and efferent arteriole) was *the only site* at which protein precipitates were demonstrated The amount of these precipitates exceeded that found in our control patients On the contrary the basement membrane which was presumably the site of the functional lesion in these patients was completely free from protein precipitates Our findings corroborate the results obtained by Drummond *et al* (3)

Whenever *proteinuria or a nephrotic syndrome was associated with haematuria* (13 pa

The Zimmer Hargrave test Rose Waaler test Latex test and antinuclear factor test were negative in all patients

*Serum complement*

Complement levels were determined in C H50 units (12) During each assay a C H50 titer was measured in a control serum This control serum consisted of a pool of fresh serum which was immediately frozen and kept in small lots at − 190 C

The C H50 titers of our patients were expressed as a percentage of the quantity in normal serum

*Renal biopsies*

25 Surgical and 5 needle (Table 1 case 11 13 17 29 30) biopsies were studied They were carried out between 3 months to 12 years after the onset of the disease

*Controls*

Renal tissue was obtained from 11 adults aged 36–71 years during urological operation Histology did not reveal glomerulonephritis only arterio and arteriolo sclerotic lesions were observed

*Histology*

Renal tissue was fixed in Zenker s fluid and em bedded in paraffin Sections cut at 1 2 μ were stained with haematoxylin-eosin (H E) periodic acid Schiff (P A S) Masson Goldner trichrome (M G T) Jones periodic acid silver methenamine (I A S M) and phosphotungstic acid haematoxylin (P T A H) (17)

Glomerular damage is indicated by the subjectively assessed degree (− to + +) of endocapillary and epithelial cell proliferation of changes of the capil lary basement membrane and of increase of the me sangial region (Table 1) All patients showed diffuse renal pathology

Sections previously used for the immunofluorescent technique were fixed in formalin macrodex and sub sequently stained with Jones periodic acid silver me thenamine/haematoxylin eosin (I A S M /H E)

*Antisera*

Antisera to human IgG IgA IgM complement (25) fibrin and albumin were prepared in rabbits The monospecificity was shown by immunoelectropho retic double diffusion and passive haemagglutination techniques In the last technique pure human antigens namely IgG IgA IgM fibrinogen and albumin were fixed to tanned erythrocytes Agglutination was ob served only on incubation of an antiserum with erythrocytes coated with the corresponding antigen Absence or presence of antibodies against comple ment factors in the antisera was established also by haemagglutination sensitized Lewis cells and erythro cytes sensitized by a natural occurring cold factor were used after *incubation with fresh serum*

The rabbit anti human IgG IgA and IgM sera were also tested on their monospecificity in our in direct immunofluorescent system using monoclonal bone marrow cells of patients with IgG IgA and IgM paraproteinemia

On the basis of the above mentioned controls the rabbit antisera employed in this study were mono specific except for the anti human albumin serum which contained additional precipitating antibodies against an α globulin On reversed immunoelec trophoresis (trough human antigen well rabbit anti serum) specific antibodies in these rabbits appeared to be present exclusively in the IgG fractions

The precipitation titers of the antisera (?) as sessed by agar blocktitration (troughs of 0 2 by 1 cm twofold antiserum dilutions wells with a dia meter of 0 25 cm and a distance of 0 4 cm from centre to trough *twofold dilutions of the antigen*) were / / / / / for the anti IgG anti IgA anti IgM anti-complement anti fibrin and anti albumin serum respectively

A sheep anti rabbit IgG and a horse anti human im munoglobulin serum were tested only by immuno electrophoretic and double diffusion techniques and as far as precipitating antibodies were concerned were shown to be specific

Rabbit antisera against the M protein of type 1 12 and 14 group A haemolytic streptococci were kindly provided by Dr C E de Moor (head Depart ment streptococcal research Rijks Instituut voor de Volksgezondheid Utrecht)

*Conjugation procedure*

Sheep anti rabbit IgG rabbit anti human Ig and rab bit anti human complement serum were coupled to fluorescein isothiocyanate (F I T C) The procedure was as follows (26) A chromatographically homo geneous IgG antibody fraction obtained by DEAE Sephadex A 50 chromatography of ammonium sul phate precipitated globulins was conjugated to F I T C using 16 mg F I T C per gram protein The conjugate was purified by DEAE Sephadex A 50 salt gradient elution method Fractions with molecular F/P ratio between 1 and 4 were pooled and em ployed in this study The precipitation titre of the sheep anti rabbit IgG reagents after conjugation de termined as mentioned above was /

*The indirect immunofluorescent technique*

Sections of kidney specimens frozen in liquid nitro gen were cut at 2 μ in a cryostat at − 15 C mounted on clean glass slides to dry for 30 minutes and then washed at 37 C in 3 changes of 0 01 M phosphate buffered 0 15 M saline pH 7 2 for 15 minutes each The sections were then flooded with a rabbit antiserum for 30 minutes at room tempera ture and washed in the same manner again After incubation for another 30 minutes with the fluores cent sheep anti rabbit IgG reagent diluted / a *final washing procedure at room temperature* was performed The slides were mounted in buffered glyc erol and observed with a Leitz fluorescent micro

1 10 ml formaldehyde 40 1 g $CaCl_2$ 90 ml macrodex (average molecular weight 75 000)



*Fig. 1* (Table 1, case 25) (*a*) To the left: Fibrin in the mesangium and in a linear pattern as well as along the basement membrane. To the right: The same section restained with P A S M / H E (Original magnification 250) (*b*) To the left: IgA present only in the mesangium. Deposits of IgM, IgG and complement were seen in a similar pattern. To the right: The same section restained with P A S M / H E (Original magnification 250)

nating from the mesangium (23). The protein precipitates were present on the outer side of these lamellae (Fig. 3). In the 2 children with low serum complement titres (cases 15 and 21) complement in contrast to the immunoglobulin was extensively fixed in the mesangium as well, mostly in a linear but sometimes also in a punctuate distribution along or within the basement membrane (Fig. 4). In case 15 IgG was seen as granules in the mesangium and within the basement membrane (Fig. 5). In case 21 we found slight mesangial IgG deposits. IgM was detected in moderate quantities in both cases in a linear distribution along or

Table 2 *Patients with only fibrin along the glomerular capillary basement membrane*

| Case Table 1 | Histopathology of glomeruli | | | | Immunofluorescence of glomeruli[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | IgG | | IgA | | IgM | | C | | Fibrin | | Albumin | |
| | Epith cell pr | Endocap cell pr | BM | Mesangial thickening | BM | MES | BM | MES | BM | MES | BM | MES | BM | MES | BM | MES |
| 29 | – | + | ± | ± | – | – | – | – | – | + | – | + | + | – | – | – |
| 30 | – | + | + | + + | | – | – | – | + | + | ± | + | + | + | – | – |
| 18 | ± | ± | ± | + | – | + | – | + | – | + | – | + | + | + | – | – |
| 16 | ± | ± | ± | + | – | + | – | + | – | + | – | + | + | ± | – | – |
| 24 | ± | + | ± | + | – | + | ± | + | ± | + | ± | + | + | + | – | + |
| 25 | | + + | ± | + | – | + | – | + | – | + | – | + | + | + | – | + |

[a] + indicates the presence of the protein – indicates the absence of the protein

tients) we found that not only were the light microscopic changes usually more severe but in 5 cases plasma protein precipitates were observed *on the basement membrane* in addition to the mesangium (case 15 16 17 18 and 19)

In 2 patients with *Anaphylactoid purpura* it was possible to demonstrate IgA IgG complement and fibrin in the mesangium and fibrin was localized also on the basement membrane In 8 of his patients Drummond (4) found IgG $\beta_1$C and fibrin only in the mesangium but mentions no results of an IgA study

The complete absence of IgG and IgA was conspicuous in sections from 5 patients with *hereditary diffuse nephropathy* A small quantity of IgM was present and complement was observed chiefly in the mesangium In the 2 patients with nerve deafness (case 29 and 30) fibrin was found on the basement membrane in the others it was found only in the mesangium We know of no previous detailed immunofluorescence study in patients with hereditary diffuse nephropathy While it is conceivable that some relation exists between the severity of the histological changes and the extent of protein precipitation, and our data (in Table 1) support this view it is our impression that the same relation is not present in patients with hereditary nephropathy In these patients we found histological changes of a degree of severity which suggested the presence of extensive immunoglobulin precipitates These precipitates however were not demonstrable

Taking all cases together a localization of protein *precipitates in the glomerular capillary basement membrane region* was established in 11 children (Table 2 and 3) All these patients showed haematuria in addition to other manifestations of diffuse nephropathy In 6 of these children (Table 2) the *protein precipitates* on the basement membrane consisted chiefly of fibrin in a linear pattern (Fig 1) A striking feature of this group was that it included both patients with anaphylactoid purpura and the 2 children with nerve deafness and hereditary nephropathy Since immunoglobulins and complement were not locally demonstrable it is hardly attractive to explain the presence of fibrin on the basis of an immunological mechanism (18)

In 5 children the protein precipitates on the basement membrane consisted not only of fibrin but also of *immunoglobulins and complement* (Table 3) These cases were histologically different from the others in the severity not *only of glomerular cell proliferation* but also of changes in the basement membrane (swelling and splitting) In patients 19 and 22 the *proteins* were localized in a lumpy bumpy distribution mainly on the epithelial side of the basement membrane (Fig 2) In patient 23 it was difficult to indicate the localization of protein precipitates because in this case there was hardly anything that could be described as a capillary basement membrane Histological examination disclosed only lamellae of basement membrane like material possibly to be interpreted as a product of regeneration or an

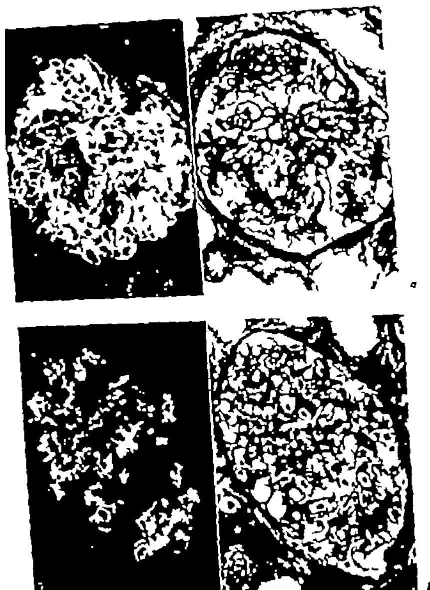


Fig 1 (Table 1 case 25) (a) To the left Fib in in the mesangium and in a linear pattern in as well as along the basem nt membrane To the right The same section restained with P A S M /H E (Original magnification 250 ) (b) To the left IgA present only in the m sangium Deposits of IgM IgG and com plement wer seen in a similar pattern To the ri ht The same se tion restained with P A S M /H E (Original magnification × 250 )

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| Case Table 1 | Histopathology of glomeruli: Epith cell pr | Endocap cell pr | BM | Mesangial thickening | Immunofluorescence of glomeruli: IgG BM | IgG MES | IgA BM | IgA MES | IgM BM | IgM MES | C BM | C MES | Fibrin BM | Fibrin MES | Albumin BM | Albumin MES |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29 | − | + | ± | ± | − | − | − | − | − | + | − | + | + | − | − | − |
| 30 | | + | + | + + | − | − | − | − | ± | + | ± | + | + | + | − | − |
| 18 | ± | ± | ± | + | − | + | − | + | − | + | − | + | + | + | − | − |
| 16 | ± | ± | ± | + | − | + | − | + | − | + | − | + | + | ± | − | − |
| 24 | ± | + | ± | + | − | + | ± | + | ± | + | ± | + | + | + | − | + |
| 25 | | + + | ± | + | − | + | − | + | − | + | − | + | + | + | − | + |

+ indicates the presence of the protein − indicates the absence of the protein

tients) we found that not only were the light microscopic changes usually more severe but in 5 cases plasma protein precipitates were observed on the basement membrane in addition *to the mesangium (case 15 16 17 18 and 19)*

In 2 patients with *Anaphylactoid purpura* it was possible to demonstrate IgA IgG complement and fibrin in the mesangium and fibrin was localized also on the basement membrane In 8 of his patients Drummond (4) found IgG $\beta_1$C and fibrin only in the mesangium but mentions no results of an IgA study

The complete absence of IgG and IgA was conspicuous in sections from 5 patients with *hereditary diffuse nephropathy* A small quantity of IgM was present and complement was observed chiefly in the mesangium In the 2 patients with nerve deafness (case 29 and 30) fibrin was found on the basement membrane in the others *it was found only in the mesangium* We know of no previous detailed immunofluorescence study in patients with hereditary diffuse nephropathy While it is conceivable that some relation exists between the severity of the histological changes and the extent of protein precipitation and our data (in Table 1) support this view it is our impression that the same relation is not present in patients with hereditary nephropathy In these patients we found histological changes of a degree of severity which suggested the presence of extensive immunoglobulin precipitates These precipitates, however were not demonstrable

Taking all cases together a localization of protein *precipitates in the glomerular capillary basement membrane region* was established in 11 children (Table 2 and 3) All these patients showed haematuria in addition to other manifestations *of diffuse nephropathy In 6 of these* children (Table 2) the protein precipitates on the basement membrane consisted chiefly of fibrin in a linear pattern (Fig 1) A striking feature of this group was that it included both patients with anaphylactoid purpura and the 2 children with nerve deafness and hereditary nephropathy Since immunoglobulins and complement *were not locally demonstrable* it is hardly attractive to explain the presence of fibrin on the basis of an immunological mechanism *(18)*

In 5 children the protein precipitates on the basement membrane consisted not only of fibrin but also of immunoglobulins and complement *(Table 3) These cases were histologically* different from the others in the severity not only of glomerular cell proliferation but also of changes in the basement membrane (swelling and splitting) In patients *19* and *22* the proteins were localized in a humpy bumpy distribution mainly on the epithelial side of the basement membrane (Fig 2) In patient 23 it was difficult to indicate the localization of protein precipitates because in this case there was hardly anything *that could* be described as a capillary basement membrane Histological examination disclosed only lamellae of basement membrane like material *possibly to be* interpreted as a product of regeneration origi



*Fig. 3* (Table 1, case 23). To the left: Fibrin present in the mesangium and on the epithelial side of membrane, the material replacing the original basement membrane. Immunoglobulins and complement were seen in the same localization. To the right: The same section restained with P.A.S.M./H.E. (Original magnification 250.)

result in linear, uniform deposition of proteins along the endothelial side of the glomerular capillary basement membrane. In many the nephritis in Goodpasture's disease and some subacute or chronic glomerulonephritides may have a similar pattern of protein deposits (20).

In this study deposits of globulins along the basement membrane could be observed in 5 children, but in only 3 cases (case 19, 22, 23) the localization was somewhat similar to the

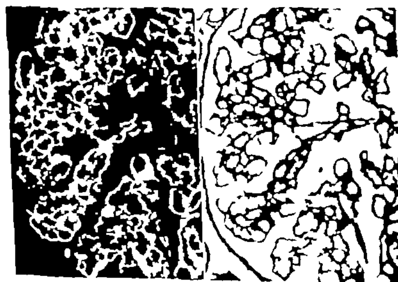


*Fig. 4* (Table 1, case 15). To the left: Complement present in the mesangium and mostly in a linear but sometimes also in a punctuate distribution along or within the basement membrane. To the right: The same section restained with P.A.S.M./H.E. (Original magnification 250.)

Table 3 *Patients with immunoglobulins complement and fibrin along the glomerular capillary basement membrane*

| Case Table 1 | Histopathology of glomeruli | | | | Immunofluorescence of glomeruli[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | IgG | | IgA | | IgM | | C | | Fibrin | | Albumin | |
| | Epith *cell pr* | Endocap *cell pr* | *BM* | Mesangial *thickening* | *BM* | *MES* | *BM* | *MES* | *BM* | *MES* | *BM* | *MES* | *BM* | *MES* | *BM* | *MES* |
| 21 | ± | + + | + + | + + | – | + | – | – | + | – | + | + | + | – | – | – |
| 15 | + + | + + | + + | + + | + | + | – | – | + | – | + | + | + | + | – | – |
| 22 | + | + + | + + | + + | + | + | – | – | + | + | + | + | + | + | – | – |
| 19 | + + | + + | + + | + + | + | + | + | + | + | + | + | + | + | + | + | + |
| 23 | ± | + + | + + | + + | + | + | + | + | + | + | + | + | + | + | – | – |

+ indicates the presence of the protein – indicates the absence of the protein

within the basement membrane but not in the mesangium (Fig 6) Fibrin deposition was not a conspicuous feature in these kidneys

The relation between human diffuse nephropathy and the conventional experimental models is not yet clear

Two major pathogenic mechanisms may be involved in immunologically induced experimental glomerulonephritis (5)

Circulating antigen antibody complexes may accumulate in the glomeruli and give rise to inflammation (2 11) This nephritis shows humpy discontinuous deposits of plasma proteins along the outer side of the glomerular capillary basement membrane A similar distribution of protein deposits may be seen in human acute post streptococcal glomerulonephritis the nephritis of systemic lupus erythematosus and in some subacute or chronic glomerulonephritide (24 14 21) In the second mechanism circulating antibodies react with glomerular bound antigens (10 27) This may

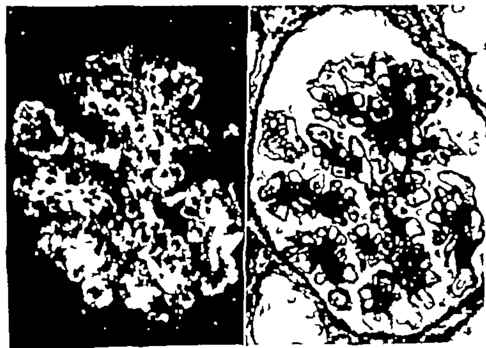


*Fig 2* (Table 1 case 19) To the left Fibrin present in the mesangium and as discrete deposits mainly on the epithelial side of the basement membrane Deposits of immunoglobulins and complement were present in the same localization To the right The same section restained with P A S M /H E (Original magnification 250) It must be kept in mind that the restaining procedure with P A S M /H E is much more illustrative than can be shown by black and white reproductions In the original slide the red coloured protein deposits are clearly visible against the black stained membrane

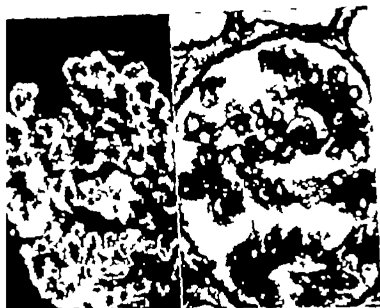


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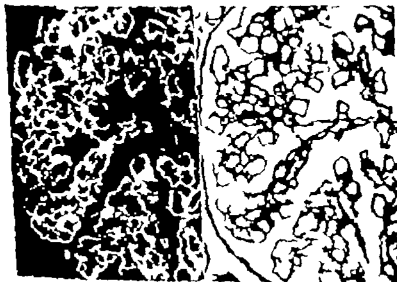


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Table 3 *Patients with immunoglobulins complement and fibrin along the glomerular capillary basement membrane*

| Case Table I | Histopathology of glomeruli | | | | Immunofluorescence of glomeruli[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | IgG | | IgA | | IgM | | C' | | Fibrin | | Albumin | |
| | Epith cell pr | Endocap cell pr | BM | Mesangial thickening | BM | MES | BM | MES | BM | MES | BM | MES | BM | MES | BM | MES |
| 21 | ± | + + | + + | + + | – | + | – | – | + | – | + | + | + | – | – | – |
| 15 | + + | + + | + + | + + | + | + | – | – | + | – | + | + | + | + | – | – |
| 22 | + | + + | + + | + + | + | + | – | – | + | + | + | + | + | + | – | – |
| 19 | + + | + + | + + | + + | + | + | + | + | + | + | + | + | + | + | + | + |
| 23 | ± | + + | + + | + + | + | + | + | + | + | + | + | + | + | + | – | – |

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within the basement membrane but not in the mesangium (Fig 6) Fibrin deposition was not a conspicuous feature in these kidneys

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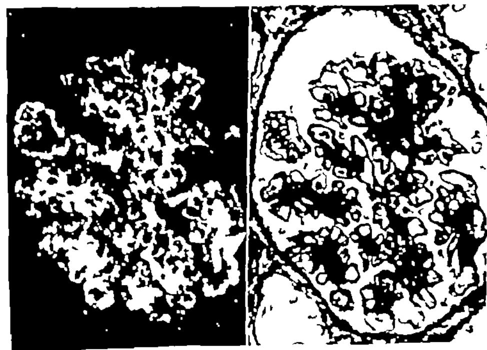


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protein in the glomeruli of these patients but not one of these met the criteria of streptococcal glomerulonephritis. Our material therefore clearly differs from that described by Segal (26) who did find streptococcal antigen in most of her patients with acute nephritis and in some with chronic nephritis.

With one exception (Table 1 case 21) the study of sera for antibodies against normal human or rat kidney was negative.

It is clear that the study of protein precipitates in biopsy material holds more promise for the future. The immunofluorescent technique may be of significance in establishing further correlations between experimental nephropathy and human pathology. It may be possible with the aid of this technique to achieve a classification more related to prognosis and treatment of choice. Long term further investigations in larger series of cases will be required before a conclusion can be formulated. For the time being it is not practicable to add the immunofluorescent technique to the routine diagnostic aids.

## SUMMARY

Preliminary report of the results obtained with an indirect immunofluorescent technique applied to the study of renal biopsy specimens.

Depositions of IgG IgA IgM complement fibrinogen and albumin were investigated in glomeruli of children with diffuse nephropathies and in adult controls. The localization of the protein precipitates was confirmed by comparing the immunofluorescent picture with the results of the P A S M /H E staining of the same sections.

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The authors are indebted to Dr W H Hijmans Institute for Rheuma research Leiden who introduced the t u ing of antisera with monoclonal bone marrow cells in the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service.

The authors wish to thank Dr K W Pondman for the determinations of serum complement M v d Giessen for the preparation of antisera H J Ruys for the performance of the restaining procedures and H Borstel for technical assistance in the immunofluorescent techniques.

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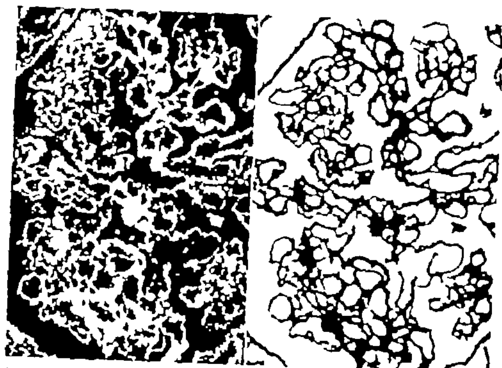


*Fig* (Table 1 case 15) To the left IgG deposits as granule in the mesangium and within the basement membrane Some linear fluorescence is also present To the right The same section restained with *P A S M / H E* (Original magnification × 250)

pattern seen in experimental antigen antibody complex induced nephritis suggesting an immune complex pathogenesis In 2 patients (case 15 21) both persistently hypocomplementic deposits of immunoglobulins and complement were seen in the basement membrane region The localization of these proteins was not exclusively linear however (Fig 4 and 5) and the resemblance to experimental antiglomerular antibody induced disease therefore less apparent

We failed to demonstrate streptococcal M

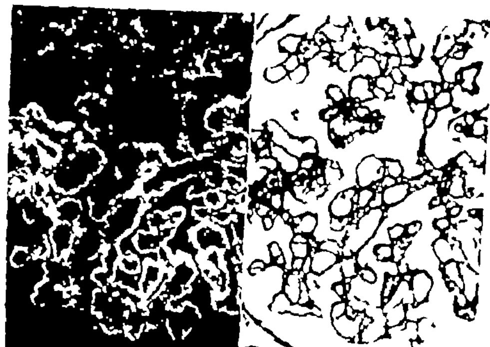


*Fig* 6 (Table 1 case 15) To the left IgM in a linear distribution along or within the basement membrane but not in the mesangium To the right The same section restained with P A S M /H E (Original magnification × 250)

*Acta Paediat Scand* 58 385–392 1969

# URINARY $C_{19}$ AND $C_{21}$ STEROID PATTERNS IN ISOSEXUAL PRECOCIOUS PUBERTY DURING LONG TERM TREATMENT WITH GESTAGENS

W M TELLER G MÜRSET and O SCHELLONG

*From the Departments of Pediatrics Universities of Heidelberg Marburg and Munster Germany and Zurich Switzerland*

Puberty is initiated by neurohumoral mechanisms which lead to the increased secretion of gonadotrophins (for details refer to Donovan and van der Werff ten Bosch (5)) This sequence of events pertains to normal as well as to idiopathic isosexual precocious puberty (i p p ) Wilkins (24) emphasized the need for a potent gonadotrophin inhibitor in the treatment of i p p Various *attempts to diminish* gonadotrophin secretion by the application of different steroids have not been entirely satisfactory Conclusive information regarding the influence of 6α methyl 17α hydroxyprogesterone acetate on steroid patterns in patients with i p p is not available Therefore the urinary excretion of 10 individual $C_{19}$ and $C_{21}$ steroid metabolites was determined before and during long term treatment with this gestagen in four boys and two girls suffering from i p p

Since Gupta & Zimprich (11) had previously emphasized the fact that patients with i p p have unusually high urinary levels of dehydroepiandrosterone we paid particular attention to the excretion of this 11-deoxy $C_{19}$ steroid

## MATERIAL AND METHODS

Two girls (B E 4 / K H 3 / ) and three boys (B H 3 / Z 3 1 / L O 6) with i p p and one boy R O 3 / ) with pinealoma were observed during the course of treatment with 6α methyl 17α hydroxyprogesterone acetate (Depo-Provera[1] MAP) or 6-chloro-6-dehydro-17α acetoxyprogesterone (Chlormadinone acetate (Gestafortin[2]) CMA) The clinical diagnosis of i p p was established by the following criteria adolescent type development of secondary sexual characteristics advanced bone and height age (10) normal neurological examination including EEG and funduscopy urinary hormone excretion (17 ketosteroids gonadotrophins) normal or slightly elevated for age no mass on rectal pelvic examination in female patients One boy (R O 3 / ) suffered from precocious puberty due to a pineal lesion This lesion was discovered only after 5 months of treatment with MAP

MAP was administered by intramuscular injections for 4 to 21 months the doses ranged from 50 to 200 mg every 10 to 14 days

Two patients (B E and Z 3 1) were subsequently treated with CMA 10 mg daily by mouth

At various intervals 24 hour urine specimens were collected for fractionation and determination of individual $C_{19}$ and $C_{21}$ steroid metabolites The method employed has been published previously (23) Briefly it consisted of the following steps β-Glucuronidase hydrolysis of the total 24-hour urine specimen extraction with carbon tetrachloride and methylene dichloride respectively solvolysis florisil column chromatography quantitative paper chromatography in different Bush type systems cm x cm elution of the paper chromatograms final colorimetric determi-

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Supported by a grant from the Deutsche Forschungsgemeinschaft, Bad Godesberg (W T)

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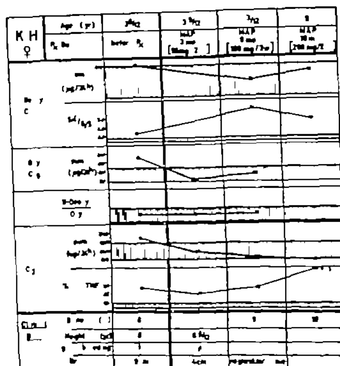


*Fig. 2.* Steroid excretion patterns and clinical data of a girl with idiopathic precocious puberty before and during treatment with gestagen. (Abbreviations and symbols as in Fig. 1.)

treatment with MAP the ratio of $5\alpha/5\beta$ of 11-deoxy $C_{19}$ steroids as well as the percentage of allo THF *increased* above pretreatment levels. The quotient $C_{19}O_2/C_{19}O_3$ remained normal.

Clinically vaginal bleeding stopped and breast development decreased. bone and height age however were not affected. The steroid excretion pattern of B. H. followed a similar trend as in B. E. and K. H. (Fig. 3). Before

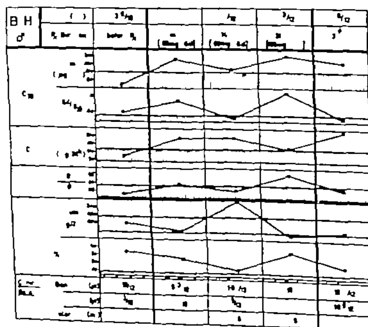


*Fig. 3.* Steroid excretion patterns and clinical data of a boy with idiopathic precocious puberty before, during and after treatment with gestagen. (Abbreviations and symbols as in Fig. 1.)

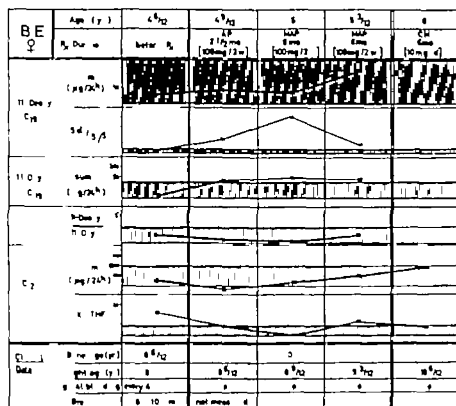


*Fig 1* Steroid excretion patterns and clinical data of a girl with idiopathic precocious puberty before and during treatment with gestagens MAP = 6α methyl 17α hydroxyprogesterone acetate CMA = 6 chlor 6-dehydro-17α acetoxyprogesterone R = treatment 5α/5β = androsterone etiocholanolone allo-THF allo-tetrahydrocortisol Hatched area = normal ranges for age as determined previously (Teller 23)

nation of the eluate of each cm of paper by micro-Zimmermann and micro Porter Silber reactions respectively

The following 10 steroid metabolites were determined Androsterone (A) (3α hydroxy 5α androstan 17 one) etiocholanolone (E) (3α hydroxy 5β androstan 17 one) dehydroepiandrosterone (DHA) (3β hydroxyandrost 5 ene 17 one) 11 hydroxyetiocholanolone (11 OHE) (3α 11β dihydroxy 5β androstan 17 one) 11 ketoandrosterone (11 OA) (3α hydroxy 5α androstane 11 17 dione) 11 ketoetiocholanolone (11 OE) (3α hydroxy 5β androstane 11 17 dione) tetrahydrocortisol (THF) (3α 11β 17α 21 tetrahydroxy 5β pregnane 20 one) allotetrahydrocortisol (allo THF) (3α 11β 17α 21 tetrahydroxy 5α pregnane 20-one) and tetrahydrocortisone (THE) (3α 17α 21 trihydroxy 5β pregnane 11 20 dione)

For reasons of shortening this communication the following steroid parameters only are reported

*11 deoxy C steroids* sum 5α/5β ratios

*11 oxy C steroids* sum ratios of 11 deoxy C /11 oxy C (C O/C O)

*C steroids* sum percentage of allo THF of the total of tetrahydroderivatives determined

## RESULTS

The results of serial steroid analyses in patients with i p p during the courses of treatment with gestagens are compiled in Figs 1–5 The previously determined age dependent normal values (mean value ± S D ) are given as hatched areas For comparison brief clinical data are presented in the lower parts of the Figs 1–5 Because of differences in results obtained by various authors (see discussion) the bone ages (10) before during and following treatment are plotted cumulatively on a simple development graph (Fig 6) Fig 1 gives the summary of findings in B E Before treatment with MAP most of the steroid parameters were within normal limits except the percentages of allo-THF During 8 months of therapy the ratio 5α/5β of 11 deoxy $C_{19}$ steroids (= androsterone/etiocholanolone) increased rather than decreased although the percentage of all THF decreased to normal range The excretion of 11 oxy$C_{19}$ steroids was slightly above normal The ratio 11 deoxy/11 oxy $C_{19}$ steroids ($C_{19}O$ /$C_{19}O_{1}$) remained normal throughout the period of investigation Regarding C steroid metabolism the change of medication to CMA was without advantage The clinical symptoms of i p p were only partially suppressed with cessation of vaginal bleeding Bone age and height age continued to advance at the previous accelerated rate Similar results as in B E were obtained in K H (Fig 2) During

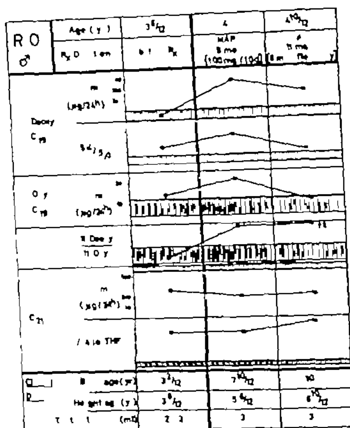


*Fig 5* Steroid excretion patterns and clinical data in a boy with sexual precocity due to a pinealoma before and during treatment with gestagen and after X ray therapy (Abbreviations and symbols as in Fig 1)

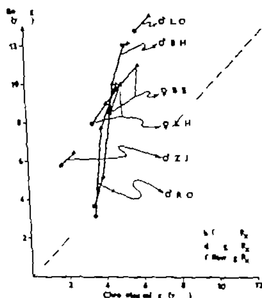


*Fig 6* Bone ages of patients with sexual precocity before (•) during (▲) and following (+) treatment with gestagens

## DISCUSSION

A decrease of gonadal and adrenal function in rats by 6α methyl 17α hydroxyprogesterone acetate was first reported by Glenn *et al* (9) These findings were subsequently confirmed by others (7 16) Recently Fekete & Szeberenyi (8) re-examined the influence of MAP on adrenal steroid secretion in rats by in vitro experiments They found reduction of adrenal weight and steroid production yet normal responsiveness to ACTH after in vivo administration of large doses of MAP Camanni *et al* (3) elicited cortisone like effects in adrenalectomized men by MAP The dose of 100 mg/die caused prompt and complete disappearance of symptoms attributable to glucocorticoid insufficiency Skinner (22) studied the influence of chlormadione acetate (CMA) on sexual development in rams and found an inhibition of follicle stimulating hormone (FSH) as well as of interstitial-

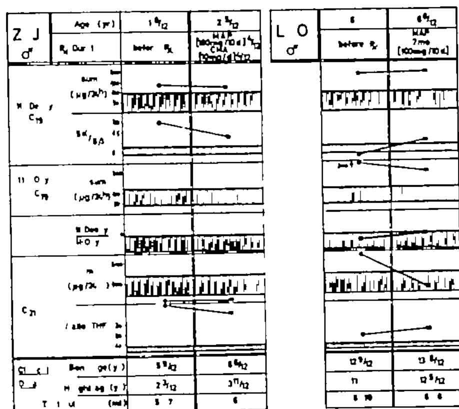


*Fig 4* Steroid excre and clinical data in two boys with idiopathic precocious puberty before and during treatment with gestagens (Abbreviations and symbols as in Fig 1)

initiation of therapy the steroid parameters were normal except increases in $5\alpha/5\beta$ of 11 deoxy $C_{19}$ steroids and percentages of allo-THF were noted During gestagen treatment the excretion of 11 deoxy $C_{19}$ and 11-oxy $C_{19}$ steroids rose above normal levels yet the ratio $C_{19}O$ /$C_{19}O_3$ increased to a pathological value only after 21 months of therapy Together with $5\alpha/5\beta$ it returned to a normal value three months after discontinuation of MAP Clinically the treatment with gestagen was without any effect The testicular size increased bone and height ages progressed at even a more rapid rate than prior to therapy

Fig 4 summarizes our findings in Z J and L O Z J had been previously misdiagnosed as adrenal hyperplasia and was treated with corticosteroids in an attempt to suppress adrenal activity He still revealed a $C_{21}$ steroid excretion below normal ranges for age 11-deoxy $C_{19}$ steroids $5\alpha/5\beta$ and percentage of allo THF as well as $C_{19}O$ /$C_{19}O_3$ were pathologically increased The treatment with MAP and CMA neither changed the steroid excretion pattern nor the clinical symptoms R O whose precocious puberty was caused by a lesion on the pineal area revealed a remarkable steroid pattern in as much as his total $C_{21}$ steroid excretion stayed well below normal ranges for age An insufficiency of ACTH secretion (perhaps due to the brain tumor) may have been the underlying reason yet this remained unproved Only during treatment with MAP the excretion of 11 deoxy and 11 oxy $C_{19}$ steroids as well as $C_{19}O$ /$C_{19}O_3$ were pathologically increased Following X ray therapy most of the steroid parameters returned towards normal During the period of study the clinical symptoms of precocious puberty remained unchanged

It was of particular interest to determine the excretion of DHA in i p p compared to normal values for age Fig 7 gives our results Before treatment five of six patients had increased DHA levels in their urines During MAP therapy no definite change occurred B E K H B H and L O showed if anything a slight *increase* rather than a decrease of urinary DHA In B H the highest value was obtained 3 months after cessation of therapy

clude from the results of our study that progesterone derivatives (gestagens) have no intrinsic influence on steroid secretion and/or enzyme systems of steroid metabolism The ratios $5\alpha/5\beta$ of 11-deoxy $C_{19}$ steroids as well as the percentages of allo-THF remained elevated above normal ranges for age From previous studies we consider these findings as evidence of androgen secretion by gonads and/or adrenals (23) Increased values of $C_{19}O_2/C_{21}O_3$ in i p p as pointed out by Blunck (2) are not in accordance with the present data since these ratios were normal in five of the six patients studied Differences in techniques and clinical material may account for this Only after initiation of MAP $C_{19}O_2/C_{21}O_3$ ratios showed an increase in two patients (B H R O) We obtained no evidence of MAP causing adrenal suppressions which is contrary to the animal experiments cited above It is interesting to note that $C_{21}$ steroid excretion was reduced in the patient who suffered from a pinealoma (R. O) One may speculate that the neoplasm had affected the hypothalamic pituitary adrenal regulatory mechanism resulting in deficient secretion of ACTH Additional cases of i p p due to pinealomas or hypothalamic tumors ought to be examined regarding their $C_{19}$ and $C_{21}$ steroid excretion patterns

## SUMMARY

Five patients (three boys and two girls aged 1 [illegible] to 6 years) with idiopathic precocious puberty and one patient (boy $3\,^{4}/_{12}$ years old) with sexual precocity due to a pinealoma were treated with gestagens $6\alpha$ methyl $17\alpha$ acetoxy progesterone (Depo-Provera®) or 6-chloro-6-dehydro-$17\alpha$ acetoxyprogesterone (Chlormadinone acetate Gestafortin®) for periods from 4 to 21 months Before and during treatment the urinary excretion of three 11 deoxy-$C_{19}$ four 11 oxy $C_{19}$ and three $C_{21}$ steroids was determined by a combined column and paper chromatographic procedure with colorimetric end points

In accordance with the failure of suppression of bone and height ages the steroid excretion patterns during treatment with Depo-Provera® remained essentially unchanged Even after 21 months of therapy the ratios of $5\alpha/5\beta$ of 11 deoxy $C_{19}$ steroids as well as the percentages of allo-tetrahydrocortisol were elevated above normal ranges It is concluded that in idiopathic precocious puberty the administration of gestagens fails to inhibit efficiently and/or persistently the secretion of gonadotrophins They continue to be secreted in amounts sufficient to stimulate endogenous androgen production

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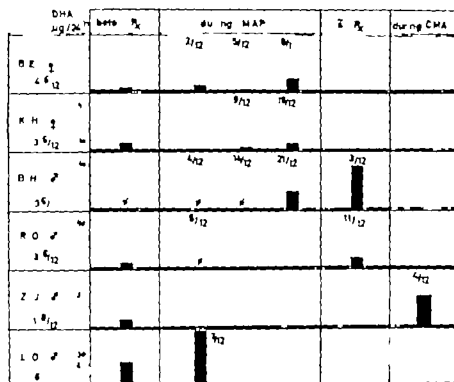



*Fig 7* Urinary excretion of dehydroepiandrosterone (DHA) in five patients with idiopathic precocious puberty and one patient (R O) with precocity due to pinealoma Values are given before during and after treatment with gestagens The duration of therapy is recorded in months at the top of each bracket For further abbreviations and symbols refer to Fig 1

cell stimulating hormone (ICSH) together with a supression of sexual development After cessation of treatment an androgenic rebound occurred Because of the promising results in animal experiments regarding gonadal suppression MAP was also used in the treatment of i p p Its oral application proved to be unsatisfactory (1) Only after a long acting intramuscular preparation (Depo Provera®) became available better clinical results were obtained Kupperman & Epstein (13) reported the cessation of vaginal bleeding reduction of skeletal maturation and normal linear growth in five girls with i p p treated with MAP for a period from 4 to 14 months Simon *et al* (21) found a depression of growth hormone release by insulin induced hypoglycemia in five normal males treated with a large (1 g) single injection of MAP

Different results were obtained by several authors (4 12 14 19 20) Most of these authors agreed that although cessation of vaginal bleeding occurred the accelerated bone maturation and growth development could not be suppressed Eberlein (6) even reported further acceleration of bone age by MAP which is in agreement with the results obtained in B H and R O (Fig 6)

Only few studies were done with regard to hormone excretion before and during treatment of i p p with MAP Lemli *et al* (15) found no decrease of urinary gonadotrophins Schoen (19) presented data indicating a suppression of gonadotrophins by MAP in three boys with i p p Simultaneous determinations of testosterone as well as fractionations of 17 ketosteroids revealed a decrease of androgen excretion New *et al* (17) reported the suppression of testosterone production by MAP in a boy with i p p Rivarola *et al* (18) found a decrease of plasma testosterone levels in three of four boys with i p p treated with intramuscular injections of MAP This therapy did not modify plasma androstendione concentrations of the patients

So far only one study has been reported with a more detailed analysis of individual urinary $C_{19}$ and $C_{21}$ steroid excretion It dealt with three patients with i p p one boy and two girls (11) During treatment with MAP for periods up to 24 months the steroid patterns did not show striking changes Similarly we may con

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SHORT COMMUNICATION

# ENZYMATIC STUDIES IN A CASE OF HEREDITARY TYROSINEMIA WITH HEPATOMA

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Hereditary tyrosinemia (1) or tyrosinosis (15) is an inborn error of metabolism which is biochemically characterized by a high blood level of tyrosine and an abnormally high urinary excretion of phenolic acids derived from tyrosine The biochemical findings are explained by an enzymatic defect resulting in an inability to form homogentisate from p hydroxyphenyl pyruvate Symptoms resembling those of acute intermittent porphyria have been observed in two cases One of these patients has been described (2) The other is the patient on whom the studies reported in this communication were performed Since then we have made the rather unexpected finding that all of six investigated patients with hereditary tyrosinemia excreted abnormally large amounts of δ aminolevulinic acid (3)

The increased excretion of δ aminolevulinic acid in patients with hereditary acute intermittent porphyria is most likely due to an increased activity of the mitochondrial enzyme δ-aminolevulinic acid synthetase (14) An increased activity of this enzyme has also been found in drug induced experimental porphyria (4 5) We have recently had an opportunity to perform enzymatic studies on a hepatoma and on liver tissues removed during surgery from a 16-year old girl with typical hereditary tyrosinemia Her urinary excretion of δ aminolevulinic acid was considerably elevated and ranged from 81 to 243 mg *per g of* creatinine In normal adults the excretion is 1 52 ± 0 59 (S D ) mg per g of creatinine (6) This patient will be reported in detail elsewhere

The activity of the δ aminolevulinic acid synthetase was seven times higher in the hepatoma tissue than in the surrounding liver and higher than the mean reported for six normal subjects by Perlroth *et al* (14) whereas the enzymatic activity in the surrounding liver was not increased (Table 1) Perlroth *et al* (14) assayed the δ-aminolevulinic acid synthetase in the liver from a fatal case of acute intermittent porphyria and found the enzyme activity to be 171 nmole per g liver per hour

Table 2 shows the results of assays for three enzymes of tyrosine metabolism carried out on the hepatoma tissue and on the surrounding liver tissue Most striking is the fact that the activity of both p hydroxyphenylpyruvate hydroxylase and L tyrosine aminotransferase

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Table 1 δ Aminolevulinic acid synthetase in hepatoma and liver in a case of tyrosinemia

| Specimen | δ Aminolevulinic acid synthetase[a] nmoles × h⁻¹ × g⁻¹ |
|---|---|
| Liver | 7 2 |
| Hepatoma | 47 1 |
| Control liver[b] | 17 3 |

[a] The biopsy specimens from the control case and from the patient were obtained during surgery immediately placed in an ice-chilled solution of 0 9 NaCl containing 0 5 mM EDTA and 10 mM TRIS buffer at 7 4 The enzymatic assays were started within 1 h after the specimen had been obtained The assay was carried out as described by Tschudy and co workers (20)
[b] The normal value based on analysis of biopsy specimens has been reported as 24 ± 5 7 (S D ) (14)

were not measurable in the hepatoma tissue whereas the isomerase was equally active in both tissues

It appears unlikely to us that acute intermittent porphyria of classical type is coexistent with hereditary tyrosinemia in our patient since no family history of porphyria has been obtained in this patient or in other cases with a combination of hereditary tyrosinemia and increased excretion of δ aminolevulinic acid Possibly these patients may have one as yet unknown biochemical defect which is genetically determined and which results in changes in tyrosine and porphyrin metabolism

Levere (11) has recently reported an increase in δ aminolevulinic acid synthetase in biopsy specimens from patients with hepatic cirrhosis In his cases there was no or only slight increase in the excretion of δ aminolevulinic acid It appears likely that the greatly increased excretion of δ aminolevulinic acid in our patient is related to an increased activity of δ aminolevulinic acid synthetase in the hepatoma tissue A relation between hepatomas and deranged porphyrin biosynthesis has previously been demonstrated in the interesting case of Tio and co workers (19) of an 80-year old woman who developed photosensitivity and marked porphyrin excretion which disappeared after the removal of a large liver tumor It is of particular interest that lack of feed back control of biosynthetic pathways has previously been demonstrated in hepatoma tissue Thus Siperstein and co-workers (16 17) have demonstrated a lack of metabolic control of cholesterol biosynthesis in several types of mouse hepatomas and in two different human hepatomas Nodular cirrhosis of the liver with active regeneration is regularly seen in subjects with hereditary tyrosinemia and a microscopic picture resembling hepatoma tissue as well as actual tumor formation has been reported (1 8 10 15) Hepatoma formation in liver cirrhosis is not rare but porphyric symptoms are not

Table 2 p Hydroxyphenylpyruvate hydroxylase (p hydroxyphenylpyruvate ascorbate oxygen oxidoreductase (hydroxylating) (EC 1 14 2 2) L tyrosine 2 oxoglutarate aminotransferase (EC 2 6 1 5) and phenylpyruvate keto enol isomerase (EC 5 3 2 1) in liver and hepatoma from a case of hereditary tyrosinemia

| Specimen | Hydroxylase µmoles h⁻¹ g | Transaminase[b] µmoles h g⁻¹ | Isomerase k/g liver |
|---|---|---|---|
| Liver | 8 8 | 14 2 | 1 52 |
| Hepatoma | 0 5 | 0 5 | 1 57 |
| Rat liver | — | — | 1 15 |
| Normal liver[d] | ≃ 30 | ≃ 20 | — |

[a] p Hydroxyphenylpyruvate hydroxylase was assayed by incubation under essentially the same conditions as used by Zannoni et al (22) p Hydroxyphenylpyruvate was determined with a modification of the enol borate method of Knox & Pitt (7)
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common in subjects with liver cirrhosis although they have been found occasionally (21). Possibly the liver disease in tyrosinemia has special characteristics, one of which would be the disappearance of metabolic control in porphyrin biosynthesis.

Since the enzymatic defect in tyrosinemia is believed to reside in an inability to convert p-hydroxyphenylpyruvate to homogentisate it is surprising to find a considerable activity of the p hydroxyphenylpyruvate hydroxylase in the liver although it is only about 25 of what has been reported for normal liver (9 18). Similarly La Du (9) reported the hydroxylase activity in liver biopsy samples from 5 cases with tyrosinemia to be between 0 4 and 4 1 $\mu$mole × $h^{-1}$ × $g^{-1}$. This would correspond to a turnover of about 2 to 20 g of p hydroxyphenylpyruvate per kg of liver per day. Maximal activities measured *in vitro* may however not necessarily be equal to the *in vivo* activity of the enzyme. The metabolic significance of p hydroxyphenylpyruvate keto-enol isomerase is not known and values for the activity of this enzyme in normal human liver are not available. In rat liver the activity of this enzyme is about 1 2 k per g of liver under the present assay condition.

The lack of p-hydroxyphenylpyruvate hydroxylase in the hepatoma tissue and the high rate of δ aminolevulinic acid synthesis in the same tissue may be purely incidental but may on the other hand suggest a biochemical relation between the two metabolic abnormalities in hereditary tyrosinemia.

## SUMMARY

The activity of δ aminolevulinic acid synthetase has been measured in liver and hepatoma tissue from a case of hereditary tyrosinemia with an increased excretion of δ aminolevulinic acid in the urine. The enzyme activity in the hepatoma tissue was seven times higher than in the surrounding liver where the activity was not increased above the reported normal range. The activity of p-hydroxyphenylpyruvate hydroxylase (EC 1 14 2 2) was about 25 of the normal in the liver tissue but not measurable in the hepatoma. Tyrosine aminotransferase (EC 2 6 1 5) activity was normal in the liver tissue but was not detectable in the hepatoma whereas the activity of phenylpyruvate keto-enol isomerase (EC 5 3 2 1) was the same in liver and hepatoma tissue.

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Since the enzymatic defect in tyrosinemia is believed to reside in an inability to convert p-hydroxyphenylpyruvate to homogentisate it is surprising to find a considerable activity of the p-hydroxyphenylpyruvate hydroxylase in the liver although it is only about 25 of what has been reported for normal liver (9, 18). Similarly La Du (9) reported the hydroxylase activity in liver biopsy samples from 5 cases with tyrosinemia to be between 0.4 and 4.1 $\mu$mole × $h^{-1}$ $g^{-1}$. This would correspond to a turnover of about 2 to 20 g of p-hydroxyphenylpyruvate per kg of liver per day. Maximal activities measured *in vitro* may however not necessarily be equal to the *in vivo* activity of the enzyme. The metabolic significance of p-hydroxyphenylpyruvate keto-enol isomerase is not known and values for the activity of this enzyme in normal human liver are not available. In rat liver the activity of this enzyme is about 1.2 k per g of liver under the present assay condition.

The lack of p-hydroxyphenylpyruvate hydroxylase in the hepatoma tissue and the high rate of δ-aminolevulinic acid synthesis in the same tissue may be purely incidental but may on the other hand suggest a biochemical relation between the two metabolic abnormalities in hereditary tyrosinemia.

## SUMMARY

The activity of δ-aminolevulinic acid synthetase has been measured in liver and hepatoma tissue from a case of hereditary tyrosinemia with an increased excretion of δ-aminolevulinic acid in the urine. The enzyme activity in the hepatoma tissue was seven times higher than in the surrounding liver where the activity was not increased above the reported normal range. The activity of p-hydroxyphenylpyruvate hydroxylase (EC 1.14.2.2) was about 25 of the normal in the liver tissue but not measurable in the hepatoma. Tyrosine aminotransferase (EC 2.6.1.5) activity was normal in the liver tissue but was not detectable in the hepatoma whereas the activity of phenylpyruvate keto-enol isomerase (EC 5.3.2.1) was the same in liver and hepatoma tissue.

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Table 1 δ *Aminolevulinic acid synthetase in hepatoma and liver in a case of tyrosinemia*

| Specimen | δ Aminolevulinic acid synthetase[a] nmoles × h$^{-1}$ × g$^{-1}$ |
|---|---|
| Liver | 7 2 |
| Hepatoma | 47 1 |
| Control liver[b] | 17 3 |

The biopsy specimens from the control case and from the patient were obtained during surgery immediately placed in an ice chilled solution of 0 9 NaCl containing 0 5 mM EDTA and 10 mM TRIS buffer at 7 4 The enzymatic assays were started within 1 h after the specimen had been obtained The assay was carried out as described by Tschudy and co workers (20)
[b] The normal value based on analysis of biopsy specimens has been reported as 24 ± 5 7 (S D ) (14)

were not measurable in the hepatoma tissue whereas the isomerase was equally active in both tissues

It appears unlikely to us that acute intermittent porphyria of classical type is coexistent with hereditary tyrosinemia in our patient since no family history of porphyria has been obtained in this patient or in other cases with a combination of hereditary tyrosinemia and increased excretion of δ aminolevulinic acid Possibly these patients may have one as yet unknown biochemical defect which is genetically determined and which results in changes in tyrosine and porphyrin metabolism

Levere (11) has recently reported an increase in δ aminolevulinic acid synthetase in biopsy specimens from patients with hepatic cirrhosis In his cases there was no or only slight increase in the excretion of δ aminolevulinic acid It appears likely that the greatly increased excretion of δ aminolevulinic acid in our patient is related to an increased activity of δ aminolevulinic acid synthetase in the hepatoma tissue A relation between hepatomas and deranged porphyrin biosynthesis has previously been demonstrated in the interesting case of Tio and co-workers (19) of an 80 year old woman who developed photosensitivity and marked porphyrin excretion which disappeared after the removal of a large liver tumor It is of particular interest that lack of feed back control of biosynthetic pathways has previously been demonstrated in hepatoma tissue Thus Siperstein and co workers (16 17) have demonstrated a lack of metabolic control of cholesterol biosynthesis in several types of mouse hepatomas and in two different human hepatomas Nodular cirrhosis of the liver with active regeneration is regularly seen in subjects with hereditary tyrosinemia and a microscopic picture resembling hepatoma tissue, as well as actual tumor formation has been reported (1 8 10 15) Hepatoma formation in liver cirrhosis is not are but porphyric symptoms are not

Table 2 *p Hydroxyphenylpyruvate hydroxylase (p hydroxyphenylpyruvate ascorbate oxygen oxido reductase (hydroxylating) (EC 1 14 2 2)* L *tyrosine 2 oxoglutarate aminotransferase (EC 2 6 1 5) and phenylpyruvate keto enol isomerase (EC 5 3 2 1) in liver and hepatoma from a case of hereditary tyrosinemia*

| Specimen | Hydroxylase[a] µmoles h$^{-1}$ g$^{-1}$ | Transaminase[b] µmoles h$^{-1}$ g$^{-1}$ | Isomerase k /g liver |
|---|---|---|---|
| Liver | 8 8 | 14 2 | 1 52 |
| Hepatoma | 0 5 | 0 5 | 1 57 |
| Rat liver | — | — | 1 15 |
| Normal liver[d] | ≃ 30 | ≃ 20 | — |

[a] p Hydroxyphenylpyruvate hydroxylase was assayed by incubation under essentially the same conditions as used by Zannoni *et al* (22) p Hydroxyphenylpyruvate was determined with a modification of the enol borate method of Knox & Pitt (7)
[b] L tyrosine 2 oxoglutarate aminotransferase was determined by a modification of the method of Lin *et al* (12)
[c] Phenylpyruvate keto enol isomerase was determined by a modification of the method of Knox & Pitt (7) with an initial substrate concentration of 0 07 µmoles of p hydroxyphenylpyruvate per ml
[d] The normal values for p hydroxyphenylpyruvate hydroxylase and L tyrosine aminotransferase are those given by La Du (9)

common in subjects with liver cirrhosis although they have been found occasionally (21). Possibly the liver disease in tyrosinemia has special characteristics one of which would be the disappearance of metabolic control in porphyrin biosynthesis.

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CASE REPORT

# A FAMILIAL 3/18 RECIPROCAL TRANSLOCATION RESULTING IN CHROMOSOME DUPLICATION DEFICIENCY (3? + − 18q −)

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A translocation may be defined as a reciprocal exchange of genetic material between two chromosomes The resulting rearrangement of the chromosome complement is often referred to as a balanced translocation Balanced translocations are not accompanied by loss of chromosomal material and have no effect on the phenotype However a carrier of a balanced translocation can produce unbalanced gametes with duplication and/or deficiency of chromosome material involved in the translocation Familial reciprocal translocations in man are generally discovered by cytogenetic investigation of these clinically abnormal individuals carrying duplication (trisomy or partial trisomy) or occasionally deficiency (deletion) of the chromosome or chromosome segments involved in the translocation The ocurrence of both types of offspring in the same family has also been reported (3 17 28) In a few instances both duplication and deficiency have been found in the same individual resulting in a chromosome complement with trisomy for part of one chromosome and deletion for part of another (4 15 20)

The purpose of the present paper is to report a hitherto undescribed example of chromosome duplication-deficiency A mother with 3/18 reciprocal translocation gave birth to a son with multiple congenital abnormalities Chromosome analysis in the child revealed trisomy for a segment of one arm of chromosome No 3 and a deletion involving the long arm of No 18 The clinical features resembled closely those reported in deletion of the long arm of chromosome No 18

## CASE HISTORIES

*Case 1* The propositus was born in March 1967 He was the second child of a 23 year-old mother and 22 year-old father Both parents were healthy The mother was No 7 among 7 siblings (Fig 1) Both her parents and two of her brothers were deceased One sister gave birth to a stillborn malformed boy Otherwise there was no known case of congenital malformation or mental retardation among near relatives and there had been no clustering of miscarriages in her family

The mother's first 3 pregnancies ended in spontaneous abortions occuring between the 8th and 12th week She then gave birth to a boy with multiple congenital malformations who died 7 weeks old (Case 2) One year later she became pregnant again The pregnancy was uneventful and there was no history of viral infection radiation exposure or drug ingestion Labour was induced one week after term At delivery the cord was tight around the child's neck and in the immediate postnatal period he was treated with oxygen and artificial respiration because of slow and inadequate breathing

The birth weight was 3710 g and the length was 49 cm The head circumference was 35 cm The facies were striking with hypertelorism epicantic folds an teverted nostrils moderately retracted midfacies prominent chin and upper lip and a downward slanting mouth ("carp mouth") The ears were normally implanted with somewhat marked antehelix There was incomplete atresia of both ear canals Examina

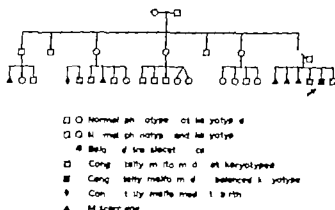


Fig 1 Pedigree of the family

tion of fundi revealed very pale optic disks The nipples were hypoplastic and wide spaced A moderate umbilical hernia was noted There was left sided drop hand and the 1st finger tended to remain in adduction while 3rd 4th and 5th fingers were kept in flection Both the hand and the fingers could be fully extended The 2nd toe on each foot was inserted slightly proximally and there was a relatively wide space between 1st and 2nd toes The scrotum was hypoplastic and the testes were not palpable The penis was rather small with a glandular hypospadias and was bound down in cordae (Figs 2 and 3) A systolic murmur grade 2 was heard best in the 2nd and 3rd intercostale spaces along the left sternal border and P was accentuated Intravenous pyelograms X ray survey of skeleton E E G urine analysis hemoglobin total and differential leucocyte counts blood urea nitrogen serum calcium phosphorus sodium and potassium blood sugar electrophoresis of serum and blood amino acid chromatography were normal

On follow up visits it became quite evident that motor and mental development were grossly retarded He never gained head control and there was a marked hypotonia At 15 months of age his height was 77 cm (25th percentile) and weight 9 6 kg (25th–10th percentile) The head circumference was 45 cm (10th percentile) The clinical course was characterized by recurrent respiratory tract infections and he died when 16 months old from bronchopneumonia

A postmortem examination revealed an atrial septal defect and a persistence of the left superior vena cava The brain appeared normal both on the surface and on cross sections

*Case 2* This boy an older brother of the propositus was born at term in January 1966 The birth weight was 2800 g and the length 48 cm The facial appearance of the child showed a striking resemblance to that of the propositus The chin and upper lip were prominent and the mouth slanted downwards The ears were normally inserted with somewhat marked helix and and antehelix There was a moderate umbilical hernia Both testes were present in the scrotum There was a slight glandular hypospadias and the scrotum encroached on the penis for the whole length of the shaft

The child died when 7 weeks old from heart failure A postmortem examination revealed a hypoplastic right lung with abnormal lobulation The right pulmonary artery originated from the descending aorta and there was an anomalous venous return to the right atrium There was an atrial septal defect and a small patent ductus arteriosus

Chromosome analysis was not performed

*Cytogenetic investigation*

Microblood cultures were set up by adding 3 drops of blood to 7 ml of tissue culture medium made up of 6 ml Difco T C 199 medium and 1 ml human AB serum 0 1 ml of phytohaemagglutinin (Burroughs Wellcome) was added and the cultures were incubated at 37 C for three days The cultures were



Fig 2 The patient 10 months old

labelled by adding 2 μc of tritiated thymidine (Radiochemical Centre Amersham specific activity 5 c/mM) five hours before termination and treated with demecolcine methyl-colchicine (Colcemid CIBA) for the last 1½ hours of culture. Well spread metaphases were photographed and their position on the slide was recorded. Autoradiographs were then prepared using a stripping film technique.

Chromosomal analysis of the propositus was performed on two different occasions. Examination of 29 well spread metaphases revealed a modal number of 46 chromosomes and the karyotype is shown in Fig 4. Ten of the metaphases were examined in detail. In each instance the karyotype contained 7 chromosomes in the D group and only 5 chromosomes in the E group. The extra D group chromosome differed in its appearance from the other members of that group in having slightly more material on the short arm. On the basis of morphological criteria the missing chromosome in the E group was tentatively identified as one of the chromosomes No 18. This was confirmed by means of autoradiography which




*Fig 4* Karyotype of propositus. The autoradiographs are placed underneath to illustrate labelling pattern at end of DNA synthesis.



*Fig 3* The facies at 10 months of age.

revealed only one late replicating submetacentric E chromosome (Fig 4).

The karyotype of the mother of the propositus is shown in Fig 5. A total of 23 cells were counted and showed a modal number of 46 chromosomes. Nine well spread metaphases were analysed in detail. Her karyotype also showed absence of a chromosome No 18 and in addition a chromosome No 3 was missing. There was one extra chromosome in the D group which was similar in appearance to that found in the propositus. In addition the mother had 17 chromosomes in the C group instead of the 16 normally expected for a female. Buccal smears obtained from the mother were those of a normal female with a single chromatin body substantiating that the extra member in the C group (No 6–12 X) was not a X chromosome.

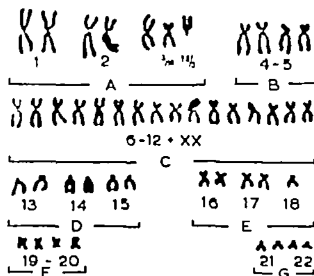



*Fig 5* Karyotype of mother of the propositus Note the two translocation chromosomes in group A

The chromosomal findings present in the mother were interpreted as resulting from a reciprocal translocation between a chromosome No 3 and No 18 The short arm and a portion near the centromere of the long arm of a No 18 chromosome became attached to a segment of one of the arms of a chromosome No 3 The resulting translocation chromosome resembled those in the D group but differed in having distinct short arm corresponding to the short arm of a chromosome No 18 (This translocation chromosome has been designated 18/3 in Fig 5) The remaining portion of the long arm of chromosome No 18 became transferred to the deleted No 3 chromosome forming a translocation chromosome resembling those in the C group (designated 3/18 in Fig 5) However this translocation chromosome could not be distinguished with any certainty among the other members of the C group but on the basis of probability it was selected among the large submetacentrics

Since chromosome No 3 has a median centromere and the two arms have similar label ling pattern on autoradiography it was impossible to decide which arm was deleted It was also difficult to give an exact assessment of the size of the terminal segments of chromosome No 3 and No 18 involved in the reciprocal translocation The absence of any phenotypic manifestations in the mother however suggests that no chromosome material was lost

The chromosomal findings in the propositus can be explained as resulting from his having received a normal chromosome No 3 and the 18/3 translocation chromosome from his mother With the contribution of another normal No 3 chromosome from the father the propositus became effectively trisomic for the segment of chromosome No 3 attached to the short arm of chromosome No 18 Since he did not receive the 3/18 translocation chromosome he was also deficient for the segment of the long arm of chromosome No 18 that was transferred to the deleted chromosome No 3

The father and one brother of the propositus 3 sisters of the mother and 4 first cousins of the propositus all had normal karyotypes (Fig 1)

#### *Serum immunoglobulin studies*

The concentration of IgG and IgM globulins was estimated by the radial diffusion plate method using specific antibodies incorporated in agar gel When the propositus was 8 months old the serum level of IgG was 15 0 mg per ml (mean normal adults 12 4±2 2) and the IgM level 1 0 mg per ml (1 73±0 35) The presence of IgA was investigated using the ring test precipitation with specific anti IgA The result indicated that IgA was present in normal concentration

Both parents and the child had blood group O The isoagglutinins were quantitated by making serial two fold dilutions of serum in saline An equal volume of 0 2 suspension of the appropriate red cells was added and agglutination determined macroscopically after 2 hours incubation at room temperature without centrifugation and after centrifugation The titrations were repeated with the same technique but after incubation overnight at 4 C Both parents had relatively high isoagglutinin titres The father had a titre of 4096 for anti A and 2048 for anti B The corresponding titres in the mother were 1024 and 512 Serum samples from the child were taken at the age of

Table I *Results of blood serum and enzyme typing*

| Marker system | Propositus | Mother | Father |
|---|---|---|---|
| ABO | 0 | 0 | 0 |
| MNSs | MSs | MSs | Ms |
| Rh | CcDee | CCDee | ccee |
| P | + | + | + |
| K | − | − | − |
| k | + | + | + |
| Le(a) | + | + | + |
| Le(b) | − | − | − |
| Fy(a) | + | − | + |
| Fy(b) | + | + | + |
| Lu(a) | − | − | − |
| Jk(a) | | + | + |
| Gm | a+x+b+f+g+ | a−x−b+f+g− | a+x+b−f−g− |
| Hp | 2−1 | 1−1 | 2−1 |
| Gc | 1−1 | 2−1 | 1−1 |
| Ag(x) | + | − | + |
| Lp(a) | − | − | + |
| Lt(a) | + | + | + |
| PGM | 2−1 | 2−1 | 1 |
| AK | 1 | 1 | 1 |
| Pi | MM | MM | MM |

K Kell k Cellano Le(a) (b) − Lewis Fy(a) (b) − Duffy Lu(a) − Lutheran Jk(a) − Kidd
Hp haptoglobins Gc group specific component Ag(x) Lp(a) Lt(a) lipoprotein
PGM phosphoglucomutase AK adenylate kinase Pi − protease inhibitor

8 and 12 months By conventional technique there was no detectable agglutination at any time With centrifugation the titre of anti B was 8 at 8 months of age and 2 at the age of 12 months In both instances the agglutination was very weak Anti A isoagglutinins were not detected with certainty in either sample

The serum sample taken at 8 months of age was treated with 2 mercaptoethanol One volume of serum diluted with an equal part of saline was mixed with an equal volume of 0 1 M 2 mercaptoethanol in phosphate buffer pH 7 4 and incubated at 37 C for two hours the mixture was then dialysed against frequent changes of buffered saline As a control serum in the same dilution was treated with phosphate buffer Following treatment with 2 mercaptoethanol anti B isoagglutinin was no longer detectable indicating that the isoagglutinin primarily present belonged to the IgM class of globulin

The results of gammaglobulin typing of serum samples from the propositus and his parents are listed in Table I There was no inconsistency in the findings

The *i* antigen in red cells was present in usual strength

*Genetic markers*

Genetic marker systems are depicted in Table I The propositus was heterozygous at the MNS Duffy phosphoglucomutase and haptoglobin loci and had inherited Rh and Gm factors from the mother thus excluding any possibility that the deleted segment might involve those loci

## DISCUSSION

Recent cytogenetic investigations have indicated that a deletion involving the long arm of chromosome No 18 is connected with a certain constellation of congenital anomalies sufficiently characteristic to allow the delineation of a new clinico-cytogenetic entity A total of 19 cases 11 females and 8 males with this

Table 2 *Clinical findings in deletion of the long arm of chromosome No 18*

The numbers indicate Frequency of the anomaly number of cases with data on this anomaly

| | Reported cases | Propositus |
|---|---|---|
| Mental retardation | 16/16 | + |
| Microcephaly and/or oxy scapocephaly | 12/15 | + |
| Hypotonicity | 12/16 | + |
| Short stature | 12/14 | − |
| Mid face retraction | 13/17 | |
| Prominent antihelix | 11/15 | + |
| Atretic or hypoplastic ear canals | 10/14 | + |
| Ocular fundoscopic anomalies | 10/16 | + |
| Hypertelorism | 7/12 | + |
| Epicanthal folds | 7/14 | |
| Carp mouth | 7/9 | + |
| Tapering fingers | 9/11 | |
| Anomalies of feet and/or toes | 10/14 | + |
| Congenital heart disease | 8/16 | |
| Widely separated and/or hypoplastic nipples | 4/7 | |
| Cryptorchidism | 5/5 | |
| Hypoplastic penis | 4/5 | + |
| Hypospadias | 1/5 | |

deletion have been reported (2 7 11 13 14 18 19 22 26 28 30) Some reports have been preliminary with incomplete clinical description The clinical findings in these reports have been included in Table 2 which summarize all available clinical data on this condition

A priori it would seem hard to predict the phenotypic manifestations of a chromosome abnormality involving both partial trisomy for one chromosome and deletion of another However comparison of the clinical features in the propositus of the present study with those reported in long arm deletion of chromosome No 18 reveals considerable similarity (Table 2) The same holds true for the older brother of the propositus in whom chromosome analysis was not performed It is rather noteworthy that the concomitant trisomy for a segment of one arm of chromosome No 3 apparently conveyed few disturbing elements to the clinical picture Familial 3/B translocation resulting in trisomy for a portion of one arm of chromosome No 3 has been described in a family with six affected infants (29) All died within a week of birth and had severe congenital abnormalities quite different from those found in the present family Since chromosome No 3 has a median centromere and the two arms have a similar labelling pattern on autoradiography it is impossible to determine which arm was involved in the chromosome rearrangement in the two families On the basis of the dissimilarity of the phenotypic manifestations it seems most probable that different arms have been present in triplicate in the affected subjects of the two families

The tetravalent configuration resulting from the pairing of translocation chromosomes and their normal homologues in the first meiotic division of the heterozygote is shown in Fig 6 The chromosomal constitution of the gametes formed will be determined by the type of segregation of the centromeres at anaphase I There are three possible anaphase disjunctions and each give rise to two types of gametes (Gamete I–VI in Fig 6) If alternate segregation occurs genetically balanced gametes will result Gamete I has a normal haploid set of chromosomes Gamete II has the translocation set but with full complement of genetic material (balanced translocation heterozygote) Adjacent −1 segregation along the vertical axis in Fig 6 and adjacent −2 segregation along the horizontal axis will produce 4 genetically different unbalanced gametes with duplication and deficiency of chromosome material In each of gametes III and IV one of the translocation chromosomes is present in addition to its recipient chromosome (homologous centromers) and in gamete V and VI one of the translocation chromosomes in addition to its donor chromosome (non homologous centromers) The fertilization of gamete VI in the diagram by an normal gamete would give rise to a zygote with a duplication (trisomy) for part of one arm of chromosome No 3 and deficiency of the long arm of chromosome No 18 as found in the propositus of the present study

The risk of abnormal offspring to the translocation heterozygote will depend on the relative frequencies of alternate adjacent −1 and adjacent −2 segregations It has been pointed out that there is a differential transmission of Down's syndrome through male and female carriers of a D/G translocation since the empiric risk for a new affected child is considerably higher for a female than a male carrier (9) A similar differential transmission has been demonstrated in a large kindred with a 3/B translocation (29) Either decreased ability of the spermatozoa containing an unbalanced chromosomal complement to initiate fertilization or a non random segregation of chromosomes during spermatogenesis may explain this phenomenon Assuming random segregation the risk of duplication-deficiency and a new affected child would be 2/3 However because of the repeated abortions in this patient's mother it may be assumed that some of the gametes will produce non viable zygotes and thus depress the risk figure for any live born baby The parents in this family were advised that the chances of having a healthy child were somewhat better than 1/3 among live borns and probably in the range of 1/2 However they were also advised that any normal appearing child would have a 50 chance of being a translocation heterozygote like the mother Faced with these facts they decided to have more children and subsequently the mother gave birth to a boy with normal male karyotype (the fertilization of gamete I in Fig 6)

Translocation heterozygosis appears to be a relatively common cause of deletion syndromes Thus it has been estimated that parental translocation carriers account for about 13 of cases of cri du chat syndrome (3) Until now a total of 4 families have been reported in which deletion of the long arm of chromosome No 18 had arisen from translocation carriers Chromosome No 4 was the other chromosome involved in the rearrangement in one family (28) a G group chromosome in another (14) and in the remaining

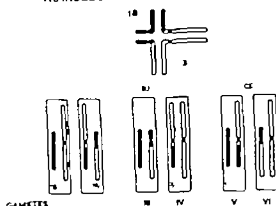



*Fig* 6 Schematic drawing showing the 6 types of gametes resulting from meiosis in a translocation heterozygote

two the other chromosome involved was not identified (2 22) In one of the families there were two affected children (14) When the present case and those from the literature are pooled the estimated frequency of translocation becomes 6/20 or 30 If one family is excluded because of the possibility that it might have been ascertained through the presence of more than one affected child the resultant frequency is 4/18 or 22 In any case this is a remarkably high frequency when compared to those found in trisomy syndromes and emphasizes the need for chromosome analysis when deletion syndromes are suspected

The virtual absence of anti A and the very low levels of anti B isoagglutinins in a one year old infant with normal serum levels of IgM and IgG globulins was a rather unexpected finding and may indicate an interference with the isoagglutinin development It now seems reasonably well-documented that the blood group antibodies the so-called natural isoagglutinins may develop during intrauterine life (27) The major portion of the isoagglutinins belong to the IgM class of globulin and do not cross the placenta However there may also be a smaller fraction of IgG agglutinins which pass the placenta to the foetus (12 23) and it is generally believed that the bulk of isoagglutinins in cord serum are of

maternal origin (12, 21 23 32) Most of the isoagglutinins derived from the mother disappears during the first 2–3 months of life Thus the titre of isoagglutinins tends to diminish during the first 3 months and then starts to rise, so that by 5–6 months of age 85 % of infants have their expected isoagglutinins (32) The remaining group of infants seems to reach this stage of development within the next 6 months (21) Quantitatively however the development proceeds with increasing titre until 7–8 years of age (8) Anti A tends to develop earlier and have a higher titre than anti B (32) Presumably the isoagglutinins originate as a result of antigenic stimulus by A and B substances in the environment rather than by genetic influence, wheras the capacity to make isoagglutinins seems to be an inherited character (1 25) Abnormalities of serum immunoglobulin development associated with deletions of chromosome No 18 have recently been reported by several investigators Absence of serum IgA was observed in two patients with long arm deletion and in one of them there was also an absence of salivary IgA (5 26) In addition absence of both serum and salivary IgA was noted in one case with Ring 18 chromosome (6) and in another both absence of IgA and very low levels of IgG (5) On the other hand there are several reported cases with Ring 18 chromosome and normal levels of IgA (24 26)

The concept of delayed maturation has been invoked to explain the retardation in formation and disappearance of certain proteins in the D trisomy syndrome Such retardation has been observed in the transition from foetal to adult forms of hemoglobin in the development of adult levels of erythrocyte carbonic anhydrase and in the disappearance of the i antigen in red cells (10 16) It is to be emphasized that these alterations do not imply a permanent disorder in normal development but rather that the attainment of mature state is delayed The abnormal pattern of isoagglutinin development observed in the present study might represent another example of such maturational delay and might be related to the trisomic condition However the possibility of an effect conveyed by the deletion of chromosome No 18 should also be considered in as much as abnormalities in the immunoglobulin development have been noted in this condition It has been suggested that the loci controlling the synthesis of immunoglobulins might be situated on the deleted chromosome segment (5) In that event, the inconstancy of the findings in different cases might be explained by difference in the portion of chromosome material lost in the deletion However the inconstancy and heterogenity of the findings might equally well be explained by non specific or indirect influence on the relevant structural or regulator genes It is apparent that conclusions should be avoided at the present stage and further studies on immunoglobulins in similar cases be awaited

## SUMMARY

Following 3 abortions a mother with 3/18 reciprocal translocation gave birth to 2 sons with multiple congenital abnormalities Chromosome analysis in one of them revealed trisomy for a segment of one arm of chromosome No 3 and a deletion involving the long arm of No 18 The clinical features resembled closely those reported in deletion of the long arm of chromosome No 18 At the age of 12 months he had normal serum levels of IgM IgG and IgA Both parents and the child had blood group O Serum from the child revealed an absence of anti A isoagglutinins and anti B isoagglutinins could only be detected with special technique wheras both parents had relatively high titres of both isoagglutinins The gene loci of MNS Rh Duffy Gm Hp and phosphoglucomutase can be excluded from the deleted part of chromosome No 18

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REVIEW ARTICLE

# HUMAN GROWTH HORMONE AND HYPOPITUITARY GROWTH RETARDATION

O TRYGSTAD

*From the Department of Pediatrics and the Pediatric Research Institute Rikshospitalet Oslo Norway*

*The words dwarf and dwarfism should be avoided in medicine From ancient times and from the literature this denomination has acquired an occult and depreciative meaning A pediatrician should aim at counteracting the use of this word and should rather speak about stunted growth or growth retardation Nanism may be used for the extreme forms of growth retardation in the same way as gigantism is used for abnormal overgrowth*

The first pituitary nanus successfully treated with human growth hormone (HGH) was reported by Raben in 1958 (44) During the last decade HGH has become increasingly important both in endocrinological treatment and research

The role of the growth hormone in intrauterine life and after epiphysial fusion and termination of growth is still unclear Pituitary growth hormone is not essential for the fetal growth and the birth weight is normal in hypopituitary nanism Growth of the fetus is not influenced by removal of both fetal and maternal pituitary glands in monkeys (21) Kaplan & Grumbach (25) observed acromegalic serum HGH levels in aborted human fetuses This high concentration of HGH was independent of the placental growth hormone which was low in the fetus compared to maternal values However maternal placental growth hormone may regulate the fetal growth through stimulation of another humoral agent

In adults no substitution therapy with HGH is needed if the pituitary gland is removed and they get no clinical sign of hyposomatotropinism except possibly a vague feeling of fatigue HGH is credited with an acute adipotropic lipidmobilizing effect, in addition to the anabolic somatotropic effect (9) This acute metabolic role may be the principal function of HGH after fusion of the epiphyses It is considered to be a stress hormone (14 53) which regulates the supply of highly energetic non esterified fatty acids from depot fat to muscular tissue and thereby reserves glucose for the central nervous system The heterogeneity of the metabolic action of HGH is furthermore emphasized by the fact that no HGH preparation has been found to be free of luteotropic (prolactin) activity and HGH has been observed to stimulate milk secretion in lactating women (30) The so-called human growth hormone seems to be composed of at least three different cores a somatotropic a diabetogenic and a lactogenic In acromegaly diabetes and lactorrhoea are frequent complications

The mechanisms by which the growth promoting action of somatotropin are mediated cannot be precisely defined as yet It promotes transfer of amino acids into cells stimulates ribosomal protein synthesis and influences the activity of a variety of enzymes The primary sites of action have not been

established One weekly injection of HGH induces a greater than normal rate of growth in hypopituitary nanism although the half life of somatotropin in blood is only in the range of 20 minutes Daughaday *et al* (6) demonstrated the presence of a serum sulfation factor which may mediate the anabolic effect of somatotropin The half life of this factor is about two days

The adipotropic–diabetogenic component of HGH has a synergistic effect with the somatotropic component in protein metabolism The adipotropin saves proteins by supplying energy for protein synthesis from depot fat thereby conserving protein and amino acids

The HGH molecule is a potent antigen in rabbits and guinea pigs But we do not know whether the somatotropic core or another part of the molecule is the antigenically active component Consequently we do not know exactly what we determine with an immunological HGH assay Demonstration of so-called HGH in blood does not always indicate the presence of a metabolically active somatotropic component (26)

In accordance with the metabolic activities of HGH ingestion of protein and infusion of amino acids raise serum growth hormone in man (47) Furthermore a decrease of blood glucose level induced by prolonged fasting exercise 2 deoxy glucose administration or insulin raises the serum level of the so called growth hormone (14 19 53 67)

*The production and release of growth hormone* from the eosinophil cells of the anterior pituitary gland are influenced by several humoral factors

A new family of polypeptide *neurohormones* produced in the hypothalamus are released into the hypophyseal portal system of veins for control of the anterior pituitary gland The growth hormone releasing factor (GRF) and the growth hormone inhibitory factor (GIF) are the hypothalamic neurosecretory substances which directly control the secretion of growth hormone (33)

*Thyroid hormones* are observed to augment the release of GRF and also to stimulate release of growth hormone from the pituitary gland directly (34) Secondary growth hormone deficiency in a child with thyrotropin deficiency has been reported (10) A primary lack of thyroid hormones causes degeneration of the eosinophil cells in the anterior pituitary gland (43) A peripheral resistance against growth hormone which may persist for several months after good response to thyroxine therapy has also been observed in hypothyroidism (1 22) However Houssey found that growth hormone administered to thyroidectomized animals was effective (17) These observations should be kept in mind in the evaluation of a therapeutical trial with HGH

*Hydrocortisone* has the opposite effect to the thyroid hormones it decreases the GRF content in hypothalamus and suppresses the growth hormone release elicited by insulin hypoglycemia (38) There has been an equivocal effect from HGH treatment of children with corticosteroid induced growth retardation (66) probably because of a peripheral antagonism between growth hormone and cortisone (37 57)

*Estrogens* decrease the concentration of HGH in the pituitary gland probably via an effect on the hypothalamus (5) Additionally estrogens inhibit the peripheral effect of HGH and reduce the cell-division (23)

*Testosterone* seems to give a potent HGH stimulus which increases the pituitary concentration of HGH (5) This may explain a reduced release of HGH at the performance of a HGH stimulation test in delayed puberty This response improves following testosterone therapy or the spontaneous puberty (31) Moreover the effect is bilateral because HGH treatment of patients with delayed puberty seems to initiate androgen production and a therapeutic trial with HGH is often successful in these patients

*Vasopressin* is related to the hypothalamic neurohormones and stimulates the HGH secretion It is also used in a HGH stimulation test (13)

*Insulin* is an important anabolic hormone and has a synergistic effect with HGH on the protein metabolism Generally speaking HGH increases the number of cells and insulin the size of the cell Salter & Best (52) observed that insulin had growth hormone like activity on hypophysectomized rats Furthermore the insulin induced hypoglycemia is regarded as the most potent stimulus for HGH secretion

## HYPOPITUITARY GROWTH RETARDATION

This is usually classified as organic or idiopathic Different organic lesions of the hypothalamic pituitary system are given in Table 1 Hypothalamic or more central lesions are often associated with isolated somatotropin deficiency sometimes combined with diabetes insipidus Lesions of the pituitary gland tend to give panhypopituitarism

Hypopituitary nanism has been considered to be idiophathic or somatic in 70 per cent of the cases (68) However we shall never rest content with this diagnosis An organic progressive process always has to be excluded by regular controls of the visual field ophthalmoscopy electroencephalography and eventually X ray of sella turcica and pneumoencephalography Idiopathic pituitary growth retardation has frequently been ascribed to perinatal injuries (18) in addition genetic biochemical abnormalities should be considered Enzymatic fragmentation processes of the pi

Table 1 *Organic types of hyposomatotropinism*

Central cerebral disorders with disturbed afferent stimulation to the hypothalamic pituitary system

*Hypothalamic*
Traumata at birth or later
Tumors
Infections
Reticuloendotheliosis (Hand-Schuller-Christian)

*Pituitary*
Congenital aplasia or hypoplasia
Acquired atrophy because of
Trauma at birth or later
Tumors (aneurism of art carotis int )
Granuloma
Reticuloendotheliosis
Infections
Hemochromatosis
Allergic autoimmune adenohypophysitis

tuitary peptide hormones will probably prove to be of pathological significance in several types of idiopathic hormone dysfunctions Regarding growth hormone there are possibilities for enzymatic dysfunction both in production of GRF and GIF and of the hormone itself Furthermore there may be enzymatic abnormalities in the release of growth hormone from the pituitary cells (27) or in the normal peripheral break-down of HGH with defective release of the active core (28) A *peripheral* end organ resistance has been proposed in pygmies (36) A serum inhibition of growth hormone like that observed for other tropins should be considered (62) and serum from two of our untreated patients were found to have HGH binding capacity Proteolytic enzymes which inactivate somatotropin and prolactin have been prepared from pituitary glands (7) and a destruction of HGH at its secretory site is possible

## DIAGNOSIS

The diagnosis of hypopituitary nanism is established on several clinical as well as laboratory characteristics The most important ones are given in Table 2

The *growth development* of the child is very important The height distance as well as the height velocity curve has to be drawn In hypopituitary growth retardation the height-canal" on the distance curve continually diverges from the 2 5 percentile (= −2 standard deviations S D ) and usually the height is less than −3 1/ S D Growth velocity is steady and about 2 cm yearly after three years of age A sudden stop in growth suggests an organic hyposomatotropinism and a craniopharyngeoma should be considered if hypothyroidism is ruled out

Compared with chronological age (CA) the bone age (BA) is always markedly retarded but higher than height age (HA) unless there is a pronounced secondary hypothyroidism Kaplan *et al* (24) gave a HA/BA ratio of 0 69 in isolated hyposomatotropinism and of 1 28 in multiple tropin deficiencies A BA/CA ratio of 2 3 in a patient suggests pituitary nanism (3) The HA/BA ratio is also of prognostic value

The *dentition* of a stunted child is of importance A marked retardation is suggestive of hypothyroidism or hyposomatotropinism In the consideration of short stature it is important to co-operate with an interested dentist, because the development of the teeth usually is advanced compared with the skeletal development and an orthodontic correction may be necessary

The *appearance* of a patient with hypopituitary nanism is often doll like with a large prominent forehead and with increased amounts of subcutaneous fat which tends to decrease under HGH treatment They have a relatively low sitting height for their stature (59) and their muscular strength is weak

Serum *protein bound iodine* (PBI) and serum *cholesterol* should be determined in growth retarded children in order to exclude a hypothyroid state A moderate secondary hypothyroidism is often observed in hypopituitary growth retardation and the serum cholesterol may be raised above 250 mg/100 ml in the absence of evidence of hypothyroidism (18) The clinical characteristics are typical in cretinism and athyreosis But hypothyroidism may start insidiously in childhood and the mental retardation may be absent A reduced

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Table 3 *Growth hormone stimulation tests*

1. *Oral glucose load* 1.5 g glucose per kg of body weight is given orally in the postresorptive phase in the morning Serum samples are obtained at 0 3½ and 4 hours
2. *Vasopressin test* 0 3 i u Pitressin (Parke–Davis) per kg of body weight maximum 10 i u is given intramuscularly in the fasting state Serum samples are obtained at 0 30 and 60 minutes
3. *Insulin hypoglycaemia* 0 05 (−0 1) i u insulin per kg of body weight is given intravenously in the fasting state Serum samples are obtained at 0 30 60 and 90 minutes
4. *Arginine infusion* 0 5 g arginine per kg of body weight (10  solution) is infused intravenously during 30 minutes in the fasting state Serum samples are obtained at 0 30 60 90 and 120 minutes
5. *Combined glucose insulin-arginine test* In the postresorptive phase in the morning oral glucose (as for 1) is given, 90 minutes later intravenous injection of insulin 0 2 i u per kg of body weight and 30 minutes afterwards arginine infusion (as for 4) Serum samples are obtained at 0 90 120 150 180 210 and 240 minutes
6. Other provocation tests have also been employed
   (*a*) Prolonged fasting often combined with physical stress Three serum samples are obtained with 1 hour intervals
   (*b*) Pseudomonas endotoxin 0 5 μg per kg of body weight is given intravenously Serum samples are obtained at 0 30 60 90 120 150 and 180 minutes

growth velocity can be the first and only symptom X ray examinations demonstrate reduced BA and an epiphyseal dysgenesis may be an additional sign

*Serum phosphorus* (P) is usually low in hypopituitarianism and it is demonstrated that somatotropin increases the reabsorption of phosphate in the renal tubuli (4) We observed the mean serum P in 22 patients to be 3 8 mg/100 ml S D 0 3 before the start of HGH treatment the level rose to a mean of 4 7 ± 0 6 (S D) mg/100 ml during the treatment

*Deficiency of ACTH* is studied by determination of the urinary excretion of 17 OHCS a water load test and the metyrapone test. If the latter test shows no increase of urinary 17 OHCS excretion an ACTH test should be performed in order to exclude primary adrenal hypofunction

*Hypoglycemia* i e fasting blood glucose below 50 mg/100 ml is not a predominant symptom in our patients with hypopituitarism since it has been observed in only two out of 23 HGH treated patients

Increased *insulin sensitivity* is a good sign in hyposomatotropinism (41 60) A prolonged hypoglycemia is more diagnostic than the absolute fall in blood glucose concentration In this institute the insulin tolerance test was originally interrupted by oral glucose thirty minutes after the intravenous infusion of insulin (0 1 i u per kg of body weight) (60) The test was interpreted as positive if blood glucose concentration decreased continuously from twenty to thirty minutes In non insulin sensitive individuals the blood sugar rises spontaneously after 20 minutes We now usually omit to give oral glucose in order to get a contemporary release of HGH provoked by the hypoglycemia During the performance of an insulin tolerance test the patients shall be given an i v infusion of physiological saline which may be substituted with glucose solution in cases of severe hypoglycemic symptoms The fasting blood glucose level should always be determined before insulin infusion At probable hypopituitarism the dosage of insulin should be reduced to 0 05 i u per kg of body weight

A reliable diagnosis of hyposomatotropinism was expected at the introduction of the *radioimmuno-assay of growth hormone* in 1962 (65) Failure of plasma growth hormone to rise after a stimulation test has often been considered to prove hypopituitarism However most laboratories have had technical difficulties with the radioimmunological HGH method The physician also has to know some technical details when evaluating the test The half life of HGH released in blood is 20 minutes and the intervals between the blood samples should be no more than 30 minutes The peak serum HGH value does not occur at exactly the same point of time in the different individuals and the total increase varies A negative test should not be trusted unless blood samples are obtained frequently over an adequate period of time and it has always to be confirmed by another test The most frequently employed HGH stimulation tests are listed in Table 3 The tests are not pathognomic because several conditions besides hypo-

Table 2 *Clinical and laboratory characteristics in evaluation of short stature*

| | Idiopathic hypopituitarism | Hypothyroidism | Delayed puberty | Genetic short stature | Low birthweight nanism[a] | Germinal cell aplasia (XO)[b] |
|---|---|---|---|---|---|---|
| Family occurrence | + – | + – | + + | + + + | + | – – |
| Birth weight | Normal | Normal | Normal | Normal | Decreased | Often decreased |
| Obvious growth retardation | Often from birth | Often from birth | At puberty | In childhood | From birth | In childhood |
| Growth velocity in childhood | 1–3 cm | 1–3 cm | 4–5 cm | 4–5 cm | 3–4 cm | 3–5 cm |
| Sexual development | Often infantile | Delayed | Delayed | Normal | Normal | Infantile |
| Clinical features | Often doll like | Present | Normal | Normal | Often present | Often present |
| Dentition | Retarded | Retarded | Less retarded | Normal | Normal | Normal |
| Bone age (BA/CA) | Retarded (2/3) | Most retarded | Less retarded (4/5) | Normal | Normal | Usually normal |
| Serum PBI | Often decreased | Usually decreased | Normal | Normal | Normal | Normal |
| Serum cholesterol | Often high ( 250 mg ) | Usually high | Normal | Normal | Normal | Normal |
| Serum phosphorus | Low (< 4 mg ) | Normal | Normal | Normal | Normal | Normal |
| Urinary excretion of 17 OHCS | Often low | Normal | Normal | Normal | Normal | Normal |
| Insulin sensitivity | Usually increased | Usually normal | Normal | Normal | Normal | Normal |
| Metyrapon test | Often abnormal | Normal | Normal | Normal | Normal | Normal |
| HGH stimulation tests | No response | Often negative | Usually normal | Normal | Normal | Normal |
| Metabolic HGH test | | | | | | |
| Retention of nitrogen | > 70 mg/kg/day | Less | Less | Less | Less | Less |
| Decrease of urinary nitrogen excretion | ~30 | Less | Less | Less | Less | Less |
| Increase of urinary hydroxyproline excretion | 60 | Less | Less | Less | Less | Less |
| Therapeutic trial with HGH | | | | | | |
| Increase of growth velocity | Marked | Questionable | Questionable | No | No | No |

[a] i.e. primordial nanism [b] Especially Turner's syndrome

patients with hypopituitary nanism are often observed to have an increased fasting blood glucose level and an increase of the body temperature under the HGH period

Thus the most important criteria for establishing the diagnosis of pituitary growth retardation are failure in rise of serum HGH level in the HGH stimulation tests and increase of nitrogen retention on a metabolic HGH test The conclusive proof is a significant increase of height velocity during a therapeutic trial with HGH

A *therapeutic trial* should be done if the metabolic test has confirmed the suspicion of hypopituitary growth retardation Particularly patients with a possible isolated hyposomatotropinism should be given this chance There is a great cyclic variation in normal growth velocity during the year and in the second quarter it is usually the double of that in the last quarter A few weeks of treatment may be conclusive in a positive trial But the trial should not be interpreted as negative unless the patient has been treated for one year and the growth velocity in this year is less than that which corresponds to the 50-percentile for the age on the *growth* velocity curve However a pituitary growth retardation may still be present. Both our HGH treated patients with an organic panhypopituitarism responded poorly to the treatment The oldest boy (CA 24 BA 15) had had a craniopharyngeoma removed at twelve years of age and the younger boy (CA 18 BA 11) has an intrasellar pituitary tumor They grew only 2 and 1 cm respectively during the first year of treatment Neither of them has serum HGH antibodies

Previously the diagnosis of hypopituitary nanism has been regarded as a diagnosis of probability which was confirmed by persisting infantilism and absence of puberty Today the diagnosis is most important because the patients can be offered an adequate treatment with HGH The identification of a panhypopituitarism is often easy and the identification of an isolated hyposomatotropinism is correspondingly difficult or impossible

*Isolated hyposomatotropinism* has previously been overlooked because of the spontaneous development of puberty The condition is not uncommon (15 59) but the diagnosis should be established only after 20 years of age because these patients often have a deficient and delayed puberty Rimoin *et al* called that syndrome sexual ateliosis (48) They studied 20 patients from nine families and the syndrome appeared to be inherited as an autosomal recessive trait No deficiences of tropins other than somatotropin were found The majority of these patients have diminished glucose tolerance and diminished insulin concentrations following insulin stimulation by glucose The insulinopenia probably accounts for their diminished glucose tolerance and may contribute to their reduced growth potential These patients often have a peculiar high pitched voice and wrinkled skin with an oldish look

Pubertal development has occurred in two out of 20 patients successfully treated for idiopathic hypopituitary nanism at the Department of Paediatrics Rikshospitalet Oslo However four more patients below 15 years of age have only hyposomatotropinism and may manifest gonadotropin deficiency Two of these patients had hypoglycemia and diminished glucose tolerance which were normalized after start of HGH treatment They grew 17 ½ and 21 cm in the first year of treatment and HGH therapy may completely change the phenotypical picture of these patients In untreated isolated hyposomatotropinism the prognosis concerning the final height is bad because of the spontaneous puberty with epiphyseal fusion and growth arrest, that occurred in two of three siblings described by Seip *et al* (54)

Martin & Wilkins (32) observed that idiopathic hypopituitary nanism was far more frequent in boys than girls Our treated group of patients consists of eight girls and 14 boys In recent years it has also been accepted that hyposomatotropinism may be hereditary and that the growth retardation often starts in infancy

The existence of *hereditary hypopituitary nanism* in man is proved and of our 22 pa

Table 4 *Reduced response in growth hormone stimulation tests has been observed in*

| |
|---|
| Hypopituitarism |
| Hypothyroidism—hyperthyroidism |
| Cushing's syndrome—treatment with corticosteroids |
| Diabetes mellitus |
| Obesity |
| Delayed puberty and development |
| Mucoviscidosis |
| Anorexia neurosa |
| Maternal deprivation —psychosocial nanism |
| Drugs (e g reserpin chlorpromazin) |
| Exogenous HGH supply |

pituitary growth retardation may lead to negative tests (Table 4) Patients with stunted growth due to hypothyroidism delayed puberty and psychosocial growth retardation often have no response to HGH stimuli and certain drugs may influence the response

A serum HGH concentration of 5 ng/ml or more during a stimulation test is considered to be normal Serum concentrations of less than 2 ng/ml are interpreted as demonstrating a negative test and values in between are doubtful The response is usually weaker in children than adults and stronger in females than males Inverse figures are frequently observed i e a fall of the serum HGH concentration under the test This may be explained by performance of the test in connection with a high spontaneous increase of serum HGH that often occurs in the postresorptive period in the morning the stimulatory mechanism may then be in a refractory period However serum HGH values of more than 5 ng/ml are always considered to demonstrate the presence of sufficient amounts of growth hormone in the child A positive test is usually regarded as unambiguous but Rimoin *et al* (48) observed normal serum levels of immunoreactive HGH following insulin and arginine in a patient with sexual ateliosis This may suggest secretion of a HGH molecule that is antigenically active but functionally impaired

A screening HGH stimulation test is often essential in the examination of a stunted child Hunter *et al* (20) suggested a glucose load and two blood samples after 3 ¹/ and 4 hours to provide a safe method for detecting released HGH In this institute the vasopressin stimulation test has been preferred (13) because the patients have to stay at the outpatient department only for one hour and a half Furthermore the vasopressin test is also a potent stimulus for ACTH secretion, and determinations of plasma cortisol besides HGH give good information concerning the pituitary ACTH reserve (64) In our hands this test has been a good screening method in both sexes Colic regularly develops after 10 to 15 minutes This is of some disadvantage but may be the stress which causes the release of HGH

A *metabolic HGH test* shall be used to confirm a negative HGH stimulation test in case of treatment with HGH In this hospital a modification of the metabolic test of Prader *et al* (41) and Wright *et al* (69) has been employed The patients get a rigidly constant diet containing ca 2 g protein per kg of body weight The 24 hour urinary excretion of nitrogen hydroxyproline and creatinine are determined After a control period of seven days the patients are given daily intramuscular injections of HGH for seven days (2 mg for patients below and 4 mg for patients above 20 kg) Plasma urea is determined in two blood samples drawn on the third and seventh day in both periods and an insulin tolerance test is performed on the last day of both periods HGH administration induces increase of nitrogen retention and decreases urinary nitrogen excretion An increased mean daily nitrogen retention of more than 70 mg/kg of body weight or a decrease of urinary nitrogen excretion of more than 30 and a significant increase of urinary hydroxyproline excretion are indicative of hyposomatotropinism Melvin *et al* (35) concluded that the metabolic response that distinguished most clearly pituitary nanism from other types of stunted growth was the greater fall of blood urea in the former mean falls being 11 3 mg and 3 6 mg per 100 ml respectively Improvement of the insulin hypersensitivity is of additional diagnostic value in hypopituitary nanism (41) Furthermore the

*Table 5. Expected and observed growth velocity in 20 patients with idiopathic hypopituitary growth retardation successfully treated with HGH*

| | No of patients | Mean age (years) | Mean growth velocity (cm/year) | |
|---|---|---|---|---|
| | | | Expected | Observed |
| Pretreatment period | 20 | 12 6/12 | 5 4 | 2 8 |
| Treatment period year | | | | |
| 1 | 20 | 13 6/12 | 5 4 | 9 7 |
| 2 | 13 | 14 10/12 | 5 0 | 5 8 |
| 3 | 9 | 15 | 4 9 | 5 3 |
| 4 | 6 | 14 10/12 | 4 5 | 5 0 |
| 5 | 3 | 15 4/12 | 4 0 | 4 3 |
| 6 | 3 | 16 4/12 | 4 0 | 3 8 |
| 7 | 2 | 18 | 4 0 (?) | 3 1 |

tients who are 15 17 and 20 years old After the initial catch up growth HGH treatment gives a growth velocity in the normal range only

Successful treatment with HGH for more than two years has been given to 13 of our patients with idiopathic hypopituitarism and their mean increase in height per year is 5 8 cm None of the patients has been treated with androgenic steroids but six needed thyroid replacement therapy The mean increase of BA for the group was just the same as that of HA and CA One patient with hypopituitarism got an increase of *BA of 10 3/* years during 7 years of treatment the contemporary increase of HA was 6 / years Previously several authors have stated *that* HGH treatment of hypopituitary nanism influences skeletal maturation less than height velocity (11 42 56 70) Our experience is that HGH treatment increases BA at least as much as HA *Prader (40)* also observed that HGH treatment of a patient with germinal cell aplasia increased the bone maturation but had no influence on *growth velocity* This is another reason why the indications for treatment of stunted growth with HGH should be strict

The catch up growth at the start of HGH treatment was best in the younger *group* of patients Below ten years of age the expected growth velocity was doubled during the first year of treatment

If sufficient amounts of HGH are available all patients with hypopituitary growth retardation and open epiphyses should get a chance with specific treatment The BA and not the CA of the patient is decisive for the start of HGH treatment which can even be initiated in the late twenties Hawley (16) reported a hypopituitary 67 year old man with BA of 11–12 years

*The dosage of HGH* in replacement therapy has varied considerably Rosenbloom (51) preferred weekly injections of 2 5–5 mg Raben (45) proposed 1–4 mg three times a week Prader *et al* (42) gave 5 mg per m of sufface area twice weekly and Tanner & Whitehouse (59) employed 10–50 mg weekly We have given 4 mg once weekly to patients with body weight below 25 kg and twice weekly to heavier ones We use ampoules containing 4 mg lyophilized HGH which is easily dissolved in physiological saline It is injected intramuscularly usually by a nurse or the parents and no untoward effects have been observed The HGH therapy is continued until fusion of the epiphyses or until a height in the lower normal range is acquired

Because of scarcity of HGH attempts have been made to split off a therapeutically active somatotropic component from animal growth hormones but the results have been disappointing because of a rapid production of serum antibodies in the patients (8 55) The amino acid sequence of HGH is given by Li *et al* (29) but the molecule is a big one and many years may pass until a synthetic HGH for clinical use is prepared unless a small polypeptide chain could be demonstrated to be an active core of HGH In the future it is possible that some HGH can be supplied from *in vitro* cultures of eosinophil cells of the anterior pituitary gland (39) The GRF will offer additional etiological informations in hyposomatotropinism and may be used therapeutically in secondary hypothalamic hyposomatotropinism *The GRF* is a small polypeptide which may be

tients eight are considered to have a hereditary type Four types of hereditary hypopituitary growth retardation in man have been described

1 Hereditary pituitary aplasia (58)
2 Hereditary pituitary nanism with hyposecretion of several tropins (63)
3 Isolated hereditable hyposomatotropinism is discussed above and one of our families has been reported in detail (54)
4 Laron *et al* (26) reported a type of nanism in some Jewish families The children had high levels of serum HGH which was considered to be defective and they responded well to exogenous HGH

Previously it was stated that the start of growth retardation in idiopathic hypopituitarism was at two to three years of age Only 12 of our 22 patients with idiopathic pituitary nanism treated with HGH had observations on measurements of length since infancy All these had retarded growth from the first year of life In eight of the other ten patients the parents had got the impression that retardation of growth started from 2–3 years of age These opinions may be wrong because growth velocity even in cases of stunted growth may be considerable in the first year of life An organic hypopituitarism should be suspected when growth retardation starts later in childhood

## TREATMENT OF HYPOPITUITARY NANISM

The supply of growth hormone for therapeutic purposes is scarce because the hormone is species specific and only primate growth hormone is active in man This makes strict demands on correct diagnosis of the pituitary growth retardation and thereby the selection of patients for growth hormone treatment Pituitary glands from other primates are even more scarce than the human, which for several years will probably continue to be the source of growth hormone for therapeutic use

All the commonly employed HGH preparations have produced serum *antibodies* and Prader *et al* (42) observed therapy resistance in 8 out of 18 treated patients However it is to be hoped that antibodies produced against one HGH preparation will not influence the metabolic response to other HGH preparations It is suggested that denatured HGH molecules initiate the production of antibodies, and the extraction of the human pituitary glands has to be done with great caution Moreover the preparation procedure must be careful in order to give a good yield of growth hormone activity According to our apprausal the method of Roos *et al* (49) satisfies both these conditions and in this institute a modification of this method has been used for seven years (61) Until now therapy resistance to Roos HGH has not been observed

In the Department of Paediatrics Rikshospitalet Oslo 24 growth retarded children have been treated with HGH for one year or more (additionally two patients have started treatment during the last six months) A negative trial has been observed in two patients one had delayed puberty and the other is considered to have a low birth weight nanism Of the 22 patients with hypopituitary nanism two have an organic hypopituitarism and do not respond to the HGH treatment The treatment has been stopped in two girls who attained normal heights (156 and 158 cm) They are still (23 and 25 years of age) sexually infantile but have responded well to stimulation with human pituitary gonadotropin (HPG) and PHG treatment may make it possible for these patients to become pregnant and beget children

The HGH treatment of the patients with idiopathic hypopituitarism has been a success and their mean growth velocities are given in Table 5 HGH antibodies have not been observed in these patients The mean growth velocity rose from 2 8 cm per year before HGH treatment to 9 7 cm in the first year of treatment with thereafter a decrease of growth velocity to about 4 cm following 6–7 years of treatment A growth velocity of more than 4 cm per year cannot be expected in these pa

Table 5 *Expected and observed growth velocity in 20 patients with idiopathic hypopituitary growth retardation successfully treated with HGH*

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easy to synthesize and it has been observed that ovine GRF is active in man (50)

Human placental lactogen is immunologically related to HGH, and is metabolically active in man (2) It promotes growth in the epiphyses of hypophysectomized rats (12) but there has been no report on therapeutic trial in hypopituitary growth retardation in man

*Thyroid replacement therapy* should according to Sobel (56) be given in all instances where HGH is administered We have been afraid of increasing skeletal maturation more than height growth and have given thyroxine only to patients with signs of hypothyroidism Thyroxine should be given with caution because it increases the rate of cortisone catabolism and may precipitate an adrenal crisis The administration should always be started at a low dosage and increased slowly

*Replacement therapy with adrenocorticosteroids* should preferably be avoided Even small amounts of cortisol will depress growth velocity None of our idiopathic pituitary growth retarded children get cortisol therapy In case of acute illness temporary administration may be necessary

*Replacement therapy with gonadal steroids* should not be given until a prepuberal height is attained because they advance skeletal maturation in excess of height growth Initially methandienon (0 04 mg per kg of body weight daily) can be given for six separate successive periods of one month alternating with an equal interval without steroid

Male patients continue with 10 mg methyl testosterone orally once or twice daily Testosterone (cyclopentyl ) propionate and testosterone enanthate may be more efficacious but often induce troublesome erections The patients will get a growth spurt development of pubic hair and enlargement of the phallus besides an increased muscular strength

Female patients may after the period with methandienon continue with fluoximesterone (orally 1–2 mg daily) combined with estrogen replacement therapy (diethylstilboesterol 0 5 mg daily) The substitution treatment is given continuously to bring about growth spurt, breast development, and growth of the uterus Following occurrence of vaginal bleeding therapy based on a cyclic fluctuation of estrogen and progesterone can be achieved with synthetic compounds The sequential principle used in fertility control (mestranol—chlormadinone) is convenient Moreover estrogens (1 mg diethylstilboesterol daily after epiphysial fusion) can be given continuously with additional progestogen (chlormadinone 2 mg daily) on the first five calendar days each month In cases of lack of pubic hair methyltestosterone (5 mg orally daily) should replace fluoximesterone Deepening of the voice development of facial hair or hypertrophy of the clitoris are indications for withdrawal of androgen therapy or for decrease of dosage

*Pitressin* should be given for control of diabetes insipidus

*Human pituitary gonadotropin* (HPG) administration appears to be a logical approach in order to awaken the gonads before the depression by replacement therapy with gonadal steroids We have added 5 mg crude HPG in the HGH ampoules for one year to one of the female patients but have no further experience

Psychologic support in accepting their condition is needed for these patients The failure of sexual development and muscular weakness are common complaints from boys and they should be informed that gonadal steroids will improve these symptoms The patients also require guidance in learning to live with their situation and especially vocational guidance

## SUMMARY

Human growth hormone (HGH) has somatotropic luteotropic and adipotropic/diabetogenic activities It is a potent antigen and serum concentrations can be determined immunologically However we do not know whether the somatotropic core or another part of the molecule is active antigenically

The secretion of HGH is influenced by humoral agents as well as by psychical and

physical stress HGH stimulation tests are negative in several states beside hypopituitarism

The diagnosis of hypopituitary nanism can be difficult, especially in isolated growth hormone deficiency which is not uncommon Clinical and laboratory characteristics in the diagnosis of pituitary nanism are discussed It is emphasized that growth retardation usually starts in infancy and that hereditary types are rather frequent The most important criteria in the diagnosis are negative HGH stimulation tests increased nitrogen retention and urinary hydroxyproline excretion during a metabolic study with HGH and the catch up growth at a therapeutical trial with HGH

Experiences in HGH treatment of 20 children with idiopathic hypopituitary nanism are given They all responded well and the mean growth velocity increased from 2 8 cm yearly before to 9 7 cm the first year on treatment Two patients with organic hypopituitarism did not respond to HGH treatment The principles for hormonal replacement therapy in pituitary nanism are discussed

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Acta Paediat Scand 58 420–429

# PROCEEDINGS OF PEDIATRIC SOCIETIES

## SCANDINAVIAN ASSOCIATION OF PEDIATRIC SURGEONS

Meeting Sept 11th–14th 1968
Skeikampen Norway

### I NEONATAL SURGERY

W Blystad (Oslo Norway) *Prematurity in general A survey*

H C Børresen & D Knutrud (Oslo Norway) *Parenteral feeding of neonates undergoing major surgery*

The physiologic basis of intravenous feeding in the neonatal period is incompletely understood In the past water carbohydrates and electrolytes requirements have been assessed in order to minimize damage due to the inevitable net catabolism associated with incomplete parenteral feeding In the present era where complete parental feeding is possible with the introduction of amino acids and suitable fat emulsions the problem is to investigate optimal rather than minimal requirements

It is assumed that the principles of normal breast feeding apply fairly closely to optimal parenteral feeding Parenteral supplies should thus normally increase linearly from the second day of life to fully cover the nutritional requirements on the 5th to 7th day of life A moderate dehydration (weight loss) will then be allowed to occur in order to counteract the danger of overhydration The slow increase of the parenteral fluid allows enzymes to be induced in time to take care of potentially toxic metabolites such as excess amino acids Abnormal fluid losses must however be fully replaced from the first day of life

150 ml human milk which is a reasonable intake per kg body weight for a resting afebrile full term neonate provides approximately 10 5 g carbohydrate (40 cal) 5 7 g fat (51 cal) 1 8 g protein (7 7 cal) the total caloric value of which is 98 7 cal But this volume of mother s milk includes only small amounts of minerals 1 mEq $Na^+$ 2 mEq K 1 mEq Mg 3 mEq Ca (60 mg) 1 4 milligram atoms P (40 mg)

The composition of mother s milk cannot at present be entirely reproduced in parenteral feeding programs mainly because only 2 g fat/kg (18 cal) can be administered intravenously as Intralipid Vitrum Even increasing the amount of carbohydrates to 13 g/kg and the amino acids to 2 5 g/kg (in place of the proteins of milk) does not provide more than about 80 cal/kg 70–90 cal/kg suffices to produce a positive nitrogen balance Prematures and infants underweight at term require 4–5 g/kg amino acids and 80–100 cal/kg to grow at a rate corresponding to the intrauterine weight gain In general the intake of water should be close to 140 ml/100 cal unless the humidity of the atmosphere approaches 100 % which reduces this amount to about 110 ml/100 cal Apart from deficits and abnormal losses major surgery appears to influence optimal requirements in one respect only it is in the authors experience advisable to adminis

ter only 1/2 of the calculated requirements of fluids nutrients and electrolytes in the first 24 hours following the operation to avoid pulmonary edema and excessive secretion in the bronchi

G Haglund & K Werkmaster (Gothenburg Sweden) *Anesthesiology and respiration A survey*

G Pettersson (Gothenburg Sweden) *Surgical aspects A survey*

I Louhimo & M Sulamaa (Helsinki Finland) *Surgery of premature infants*

Operative treatment of premature babies is still an unsolved problem in pediatric surgery This fact is well established by the report following which presents an analysis of neonatal surgery performed at the Children s Hospital University of Helsinki over the last ten years

Of 571 babies operated under two weeks of age in 1959–68 118 (20 7 ) weighed 2500 g or less at birth Seventy three (61 9 ) of these died during their first admission while the mortality of full term neonates was 25 4

For further analysis the series was divided in two groups the cases with and without atresia of the digestive tract Table 1 shows the results of surgery in the group without atresia Of 194 patients only 15 were premature (7 7 %) This is not much more than the over all incidence of prematurity in Finland

The picture is quite different in the atresia group (Table 2) Of these 27 3 were premature—that frequency decreasing with the level of obstruction The table also shows that more than a half of all prematures needing surgery during their first 14 days of life were patients with oesophageal atresia

Table 3 shows the results of oesophageal atresia surgery before and after the start of intensive therapy unit in 1964 There is a definite improvement in the group of full term infants Mortality decreased from 41 6 % to 24 4 During the last five years only three of the 51 full term babies with oesophageal atresia died from this di ease alone In even other deceased cases there were other major malformations as well

The results of the surgery of premature babies with oesophageal atresia have not improved with better facilities for postoperative care In 1959–63 the mortality was 65 8 in 1964–68 73 1 There was a significantly

Table 1 *Neonatal surgery 1959–68 (Non atresia group)*

| | | Full term | | Premature | |
|---|---|---|---|---|---|
| | All | Cases | Deaths | Cases | Deaths |
| Congenital clubfoot* | 46 | 44 | 0 | 2 | 0 |
| Spina bifida cystica | 25 | 24 | 3 | 1 | 0 |
| Omphalocele | 13 | 9 | 3 | 4 | 3 |
| Diaphragmatic hernia | 41 | 39 | 15 | 2 | 1 |
| Malrotation | 25 | 23 | 4 | 2 | 0 |
| Mb Hirschsprung (high) | 7 | 7 | 6 | 0 | 0 |
| Ductus omphalomesent + + intestinal duplicat | 8 | 8 | 1 | 0 | 0 |
| Miscellaneous | 29 | 25 | 7 | 4 | 2 |
| | 194 | 179 | 39 | 15 | 6 |

Since 1964

Table 2 *Neonatal surgery 1959–68 (Atresia group)*

| | All | Full term | | | Premature | | |
|---|---|---|---|---|---|---|---|
| | | Cases | Deaths | | Cases | Deaths | |
| Oesophageal atresia | 195 | 131 | 43 | 32.8 | 64 | 44 | 68 8 |
| Duodenal atresia or stenosis | 64 | 45 | 9 | 20 0 | 19 | 11 | 57 9 |
| Jejunal and ileal atresia | 38 | 28 | 15 | 53 6 | 10 | 6 | 60 0 |
| Rectal or anal atresia | 80 | 70 | 9 | 12 9 | 10 | 6 | 60 0 |
| | 377 | 274 | 76 | 27 7 | 103 | 67 | 65 0 |

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The diseased kidneys were removed at the time of the transplantation The donor was the father or the mother depending on the results of the compatibility tests In 3 cases the kidney could be attached by the usual technique In one case the renal vein was sutured to the common iliac vein In all cases it was quite easy to adapt the transplant into the pelvis

All transplants started excretion almost immediately and the creatinine level was on the second day below 1 mg per 100 ml One patient died during the fourth post-operative week After profuse intestinal bleedings and later on duodenal and gastric ulcers and cardiac arrest she died 3 weeks following resuscitation of peritonitis after perforation of the ulcers

The 3 surviving patients have now been followed up 15 7 and 4 months Two patients had slight rejections at 1 and 2 months respectively after transplantation The hypertonia and the retinal changes disappeared Due to the immunosuppressive treatment the patients have a slight osteoporosis During the short follow up no increase in length has been noticed On the other hand their general condition is very good

The patients are active live at home continue attendance at school etc Continued immunosuppressive treatment is necessary Periodic routine controls are made in the domicile hospitals

It could be said that it is possible to transplant kidneys at least in older children In the literature there are reports on children who after kidney transplantation have grown up and even born children of their own

## III FREE PAPERS

P Fogh Andersen (Copenhagen Denmark) *Congenital defect of scalp and skull Reconstruction during the first day of life*

Without treatment severe defects of skull and scalp usually prove fatal during the first weeks of life due to hemorrhage or meningitis

A premature male infant, weighing 1500 grams was born with a large defect of the skin galea and bone of the vertex only a thin transparent membrane covered the superior sagittal sinus Transferred to Diakonissestiftelsen s Hospital 10 hours after delivery it was decided to perform a primary repair of the defect by means of two rotation flaps of the entire scalp Uneventful healing followed the reconstruction and later complete bony union occurred verified at X ray

In addition the patient had a severe penoscrotal hypospadias repaired according to Denis Browne s technique and congenital hypoplasia of the abdominal muscles No hydrocephalus developed but remarkable mental retardation has been observed at follow up the child is now 8 years old

Arvid Frank Jørgensen & Ole Henrik Nielsen (Copenhagen Denmark) *Congenital Diaphragmatic Hernia*

An analysis of 74 cases of diphragmatic hernia in infants is presented Hiatal hernias are excluded

The study comprises 65 clinical cases and 9 autopsy cases collected from maternity and neonatal departments The latter infants were either stillborn (2 cases) or death was so rapid that no treatment could be instituted

12 * of the infants had a birthweight <2500 g this group had a somewhat higher mortality As expected the highest mortality was found in the group of children with large posterolateral defects of the left diaphragm There are 49 children in this group and they all had early and servere symptoms of respiratory distress 25 of them did not survive The lungs of the dead children were very small The microscopic findings suggest compression atrophy rather than hypoplasia On analysis the authors have found that the stomach was situated in the abdomen in 23 of 24 survivors while it was situated in the thorax in 21 of 25

*Table 3 Oesophageal atresia*

| | 1959–63 | | | 1964–68 | | |
|---|---|---|---|---|---|---|
| | Cases | Deaths | | Cases | Deaths | |
| Full term | 80 | 33 | 41 3 | 51 | 10 | 24 4 |
| Premature | 38 | 25 | 65 8 | 26 | 19 | 73 1 |
| | 118 | 58 | 50 0 | 77 | 29 | 37 7 |

greater number of other major malformations in the latter group of patients and that probably accounts partly for the lack of improvement However the incidence of other major malformations in all the deceased premature oesophageal atresia patients during the last ten years was 40 9 % while the corresponding figure for full term babies was almost as high 39 5 Thus the fact remains that prematurity alone still is one of the major challenges in the surgical care of neonates

Bjorn Henrikson & Gustaf Pettersson (Gothenburg Sweden) *Oesophageal atresia Operative treatment in the Children s Hospital Gothenburg 1947–67*

In the period 1947–67 127 patients with oesophageal atresia were treated at the Children s Hospital 36 patients were from Gothenburg and were 0 3 of all live births

The sex distribution was 75 boys and 52 girls (59 and 41 % respectively) and the difference indicates the tendency for male domination

Additional congenital anomalies were seen in 47 cases many of these having several anomalies simultaneously The most common types were ventricular septal defects (8) rectal agenesis (8) and patent ductus arteriosus (7) In 23 cases there was anomaly of the heart or great vessels in 16 cases the gastrointestinal tract was involved, head and face in 9 cases in 3 cases vertebral anomalies and in 3 cases mongolismus

The birthweight was under 2 kg in 24 cases, 2–2 5 in 21 2 5–3 in 37 3–3 5 in 30 3 5–4 in 13 and over 4 kg in 2 cases

The operative technique was initially that of Haight with extrapleural dissection later a two step performance with tracheostomy and gastrostomy and then an oesophageal anastomosis Currently immediate anastomosis is made in all cases where it is possible In other cases several step or immediate colon anastomosis were made

Overall mortality was 58 in the 106 anastomosed cases 51 in the 80 cases without severe anomalies 41 in the 73 cases without anomalies 37 Mortality in babies over 2 5 kg in this group has decreased from 62 through 35 to 6 during the period studied

## II SYMPOSIUM ON ORGAN TRANSPLANTS

Lars-Erik Gelin (Gothenburg, Sweden) *Kidney transplantation*

Carl Gustav Groth (Stockholm Sweden) *Liver transplantations*

Erik Thorsby (Oslo Norway) *Transplant antigens and tissue typing*

Olle Jacob Malm (Oslo Norway) *Immunosuppressive treatment*

B Lindstrom & O Lindfors (Helsinki Finland) *Kidney transplants in children*

The first kidney transplantation in Finland from a living donor was performed in 1964 By september 1968 36 transplantations had been performed on 35 patients 4 of them children 11 11 13 and 14 years of age (2 boys and 2 girls)

The transplantations on children were induced by chronic kidney failure due to nephrocalcinosis (caused by vitamin D) in one case and chronic glomerulonephritis in 3 cases In two cases the general development of the children was retarded by the disease In all 4 cases the disease was in a terminal state with severe uremia disturbances of the electrolyte level and hypertonia including changes of the retina In the 14 year old girl puberty was delayed

of the investigations progress have been presented

In the first three days postoperatively the fibrinogen concentration in both adults and children rose 200–300 mg and remained high for more than 1 1/2 weeks The preoperative concentration was considerably higher in older adults than in children The blood suspension stability in many of the children was lower than in the adults

All patients showed a rapid rise in activity of antihaemophilic globulin A

The preoperative platelet level was slightly higher in children Following a moderate thrombopenia in most of the adults but only a few children postoperative thrombocytosis developed

In studying platelet properties many methodological problems are involved The level of adhesive platelets seems to change little with the age of the patient but the ADP induced adhesiveness is often more rapidly counteracted in children

No fibrinolytic activity was seen on unheated fibrin plates (mainly measuring plasminogen activators) after the first postoperative day Proteolytic capacity (maximal activation of plasminogen by urokinase) usually showed a marked drop on the 2nd and 3rd days followed by a slow increase This may mean considerable plasminogen consumption not only during surgical procedure but also in a more generalized postoperative subclinical intravascular coagulation

E Enger S Hagberg H Haljamae & H Rockert (Gothenburg Sweden) *Clinical application of methods for the determination of intracellular potassium*

Studies of the intracellular potassium content with ultra micro methods on single cells have been performed Single skeletal muscle cells from biopsies on experimental animals and human subjects were dissected 70–100 micron long pieces were cut from central parts of uninjured cells The dry mass was determined by X ray absorption After extraction for 2 hours with 70 nanoliters of quartz re-destilled water under liquid paraffin potassium and sodium analyses were performed on nanoliter samples of the extract by ultramicro flame photometry

With these micro-methods it was possible to determine changes in ultracellular electrolyte content under various experimental conditions In vitro metabolic changes of single cells taken before and after different pathological conditions e g haemorrhagic shock hypothermia etc could also be determined

The results from clinical applications of these methods showed that it was possible to determine electrolyte changes in single human skeletal muscle cells Thus significantly lower intracellular electrolyte values were obtained in a group of pediatric patients suffering from hypokalemia as compared to control patients

It was thus possible to follow up in vivo and in vitro electrolyte changes of single cells The clinical applications of these methods seems valuable in recording the effects of various pathological conditions and to evaluate therapeutic response at the cellular level

A Danielsen & O Knutrud (Oslo Norway) *Surgical treatment of funnel chest*

Some 39 boys and 19 girls were treated for funnel chest, 74 surgically The average age at operation was 7 years 55 % were asymptomatic None showed any serious respiration or circulation disturbance The operations were performed according to Sulamaa The authors suggest that calculation of the frontosagittal index described by Backer and co-workers seemed to be a valuable adjunct to the clinical assessment of the degree of the deformity and may also be useful as a comparison of the pre and postoperative condition

(To be publ in Zeitschrift fur Kinderchirurgie)

Th Ehrenpreis N O Ericsson & A Liwadius (Stockholm Sweden) *Urological Anomalies in Patients with Hirschsprung's Disease*

There are few reports on the incidence of urological anomalies in conjunction with Hirsch-

dead infants Moreover 3 of the 4 children in the group with an intraabdominal stomach died from surgical complications not from respiratory insufficiency It is suggested that death was due to inability of the lungs, particularly the left lung to expand and that an important factor was the higher degree of foetal intrathoracal compression exerted by the stomach

The therapeutical problem appeared to be the maintenance of normal oxygenation until the lungs were able to expand

The possibility of extracorporeal oxygenation of the blood is proposed

I Louhimo (Helsinki Finland) *Peritoneopericardial hernia*

About 40 cases of peritoneopericardial hernias traumatic or congenital have been described in literature Less than a half of them children One new succesfully operated case is presented

The patient is a son of a medical student born in February 1967 as a non identical B twin Nothing distinctly abnormal except a slight tremor was noted immediately after birth and at the age of two weeks the patient was discharged as a normal baby His weight gain however was slower than that of his twin sibling and slight cyanosis at feeding and crying was also soon noted The attacks of cyanosis became gradually worse and sometimes ended in apparent collapse with slow pulse and pale skin On examination at the age of six weeks the father heard bowel sounds in the chest and an X ray was taken This revealed a large diaphragmatic hernia mainly on the right side of the chest and surrounded by a sack Viewed laterally the route of the intestines through the diaphragma could be clearly seen to be just behind the sternum although this was not recognized at the time

The child was admitted to the Children's Clinic University of Oulu and his condition rapidly became worse An emergency laparatomy was performed through a right paramedial incision on the 28th of March, 1967 When the abdomen was opened a partial absence of the right rectus muscle was noted The right lobe of the liver most of the small intestine and part of the colon were found to be displaced through a 5×5 cm defect into an enormously enlarged pericardial cavity There was no hernial sac and after replacement of the abdominal organs the patient's heart was clearly seen through the defect The pleura was intact

It was not possible to close the large defect by direct suture without distorting the inferior vena cava and so a part of the upper posterior wall of the rectus sheath was turned backwards and upwards to help cover the defect A part of the excessive pericardium was also used for this purpose The abdomen was closed in layers without difficulty

The postoperative course was without incident Chest X ray taken two months after the operation was normal, but one year later a slight bulging was seen at the right pericardiophrenic border This is probably a part of the liver since barium meal examination revealed the bowels in the abdominal cavity The child is symptomless and developing normally so that no further surgery is planned

Peritoneopericardial defect has also been described as a part of a syndrom which additionally includes defects of the supraumbilical abdominal wall and lower sternum and some kind of congenital heart disease The partial absence of the right rectus muscle was therefore an interesting finding The sternum and heart of this patient are normal

Johs S Rø (Oslo Norway) *Postoperative haemostasis in children*

Thromboembolism is very seldom seen in paediatric surgery Postoperative studies on coagulation and fibrinolysis have therefore been undertaken on children and adults The children examined were mostly undergoing operations on the urinary tract (average age 7–9 years)

Following a review on the mechanism of haemostasis and thrombogenesis the results

ti ulitis in 13 and cancer in 1 Ectopic gastric mucosa was usually found in ulcer patients and sporadically in others The average age in intestinal obstruction was 12 years in diverticulitis 31 years Only the children had melena which started in half of them before 2 years of age

Several operative techniques were used In 8 intestinal resection was performed in 16 wedge shaped resection and in 37 different forms of resection at the base of M d In the latter group two children had postoperative intestinal obstruction one with a rapidly fatal outcome In 3 melena patients with resection at the base ectopic gastric mucosa probably remained Most diverticles in this series were broadbased In diverticulectomy stenosis must be avoided and all pathological and ectopic tissue removed Wedge shaped resection seems to be the method of choice

An apparently normal M d should be removed because disease may develop later This happened in 4 patients of the series on which laparotomy had been performed

Ch v Hedenberg & N O Ericsson (Stockholm Sweden) *Meckel s diverticulum Risks and operative indications*

Over a period of 15 years Meckel s diverticulum M d ) was found in 105 patients 92 were excised by the conventional technique Th MD was the direct source of disease in 26 in 64 an incidental finding

Routine histologic examination revealed heterotropic ventricular or pancreatic tissue in 19 cases 17 of which were diagnosed at operation The existence of heterotropic tissue was the main risk in the present series 11 out of 19 M d were the direct cause of symptoms three with perforation were due to peptic ulceration

Diverticulitis occurred in eight cases in three with perforation of the M d Fixation of the M d by a band to the abdominal wall was found in eight patients in four causing intestinal obstruction Intussusception caused by M d was present in two patients both under four years of age

There was no mortality in the series Ten complications occurred in 95 patients treated by diverticulectomy intestinal obstruction in eight subileus and an ileocutaneous fistula in one each All were successfully operated secondarily

The high rate of complications may provoke discussion on the operative indications in cases with M d We feel that innocent and incidentally found M d can be ignored while M d with heterotopic tissue of some notable extension bands or a very narrow base should be removed The presence of heterotopic tissue involving a certain area of the MD can be diagnosed at operation Small degrees of heterotopy are less dangerous

Johs S Rø (Oslo Norway) *Complications following extirpation of Meckel s diverticulum*

Intestinal obstruction following extirpation of Meckel s diverticulum was seen in three of 79 patients from the surgical departments Haukeland Hospital Bergen and the surgical department B State Hospital Oslo Adhesions were the cause in one boy who had a new laparotomy performed one month after an operation for volvulus In three patients stenosis developed at the site of diverticulectomy Two of them had symptoms in the immediate postoperative period One of them died In the other the obstruction contributed to the fatal outcome Four years postoperatively rectal bleeding occurred in the third patient Laparotomy revealed stenosis with a bleeding ulcer

In a three month old child disruption of the abdominal wound developed 5 days after a desinvagination procedure Four years after intestinal resection with side to side anastomosis another child began to bleed from a blind loop

Six out of the 79 patients therefore had complications Wedge shaped resection would possibly reduce the frequency of stenosis at the site of resection

sprung s disease and the incidences vary con siderably The incidence of urinary anomalies in Hirschsprung s disease is, apart from its theoretical interest also of practical value to the surgeon The paucity of contributions and the controversial views prompted the present study

Urologic investigation was performed on 62 patients with histologically confirmed rectocolonic aganglionosis The diagnostic procedures included urinanalysis urine cultures and sensitivity tests intravenous pyelograms and mic turition urethrocystograms

Urinary tract anomalies were encountered in 14 out of 62 patients 22 6 % Six of them had urinary tract infection two with a history of recurrent pyelonephritis Eighteen anoma lies were found vesicoureteral reflux and hy dronephrosis being the most common and af fecting predominantly the left side

No proof has yet been presented that these anomalies are of neurogenic etiology Not a single case of megacystis was found In six pa tients the anomalies affecting the upper urinary *tract disappeared after rectosigmoidectomy* This suggests that they were secondary to pro longed rectosigmoid obstruction with fecal im paction When these six patients are excluded the remaining eight constitute 13 % of the se ries still making urological anomalies the most frequent in combination with Hirschsprung s disease

The majority of urinary tract anomalies in the present series were asymptomatic This fact indicates the necessity of routine urologic investigation in patients with Hirschsprung s disease The investigation should include intra venous urography and micturition urethrocys tography

Eivin Struve Christensen & Jørgen Lejerstofte Jensen (Hellerup Denmark) *Inguinal Hernio tomy in Infants and Children*

A series of inguinal hernioplasty performed on 480 infants and children is presented 88 were boys and 12 % were girls Late follow up data were obtained from 445 (92 ) patients

Unilateral hernia was seen in 443 (93 %) patients and in 37 (7 % ) bilateral hernia was present

The clinically palpable hernia was located on the right side in 291 patients (61 %) and on the left side in 152 patients (32 %)

The most common symptom was inguinal lump seen in 423 patients

Incarceration which occurs in over 50 % within the first three months presents a special problem

In infants and young children the hernial sac is identified and carefully freed to the ex ternal ring where it is ligated

In older children the sac is removed after high ligation

Bowel resection was not necessary Two per cent (9 patients) returned with recurrence of hernia

414 patients were followed up from one to seven years following the operation for a uni lateral inguinal hernia In 41 10 % group a *subsequent contralateral* inguinal hernia devel oped

The possibility of damage to the vas defer ens and testis during routine contralateral ex ploration of juvenile hernias is mentioned

The authors are of the opinion that the small number of the series requiring a second operation does not justify a routine contralat eral exploratory operation when only one clini cal hernia exists

(To be publ in Zeitschrift fur Kinderchirurgie)

Johs S Rø & Kåre Ohma (Bergen Norway) *Meckel s diverticulum Surgical considerations*

A review is presented of 68 patients from Haukeland hospital Bergen with Meckel s di verticulum (M d ) Disease involving M d oc curred in 30 males and 13 females 25 under 15 years of age Intestinal obstruction was seen in 15 including two cases of volvulus ulcus pepticum in 14 (12 with massive melena) diver

transfusions were given frequently to 54 out of 103 children It is therefore important to try to reduce the frequency of transfusions As some of the transfusions were unnecessary more appropriate indications would reduce such unnecessary transfusions

Antifibrinolytic treatment may also reduce the blood loss following inhibition of the formation of the fibrinolytic enzyme plasmin The activating action of the urinary enzyme urokinase or plasminogen is counteracted Treatment with AMCA (trans-4 aminomethylcyclohexanecarboxylic acid) was therefore tried in a series of 10 children (with 12 controls) for a week following reimplantation of ureter

The preoperative blood loss was on average redu ed from 40 to 20 ml Postoperatively it was reduced to one quarter from 84 to 20 ml In two AMCA treated patients however renewed bleeding occurred when the treatment was stopped In six of them long greyish clots were passed with the urine from the 4th to the 9th day after operation in two of them leading to transient bladder retention

Antifibrinolytic treatment therefore seems to inhibit lysis of cloths necessary for local haemostasis in the urinary tract It also inhibits the appropriate lysis of clots in the urine

The uncertainty accompanying a treatment affecting the normal fibrinolytic balance however calls for caution in the use of antifibrinolytic treatment to reduce blood loss in uncomplicated urinary tract surgery in children

N O Ericsson & S Soderlund (Stockholm Sweden) *Compression of the trachea by the innominate artery*

An anomalous innominate artery can produce respiratory distress in infancy When the artery branchs from the aortic arch more to the left than normal it crosses over the trachea wich may be compressed

The malformation is rather frequent but very little discussed

The signs and symptoms vary with the degree of compression Some patients are asymptomatic others have slight symptoms while some have severe respiratory difficulty The symptoms are noticed from birth stridor cough and sometimes cyanotic spells The child is prone to recurrent chest infections

For the diagnosis plain roentgen films suffice The true lateral projection demonstrates an identation in the anterior wall of the trachea below the jugular fossa Angiography is not necessary The differential diagnosis does not often present any serious problems

Surgical treatment is indicated only in patients with severe symptoms Others seem to improve with age Gross described an operative procedure left sided thoracotomy and attachment of the artery to the sternum thus relieving the trachea from compression

Six cases from the paediatric clinic at Karolinska Ho pital representing different degrees of compression were demonstrated Three patients aged 5–7 months were operated on according to Gross with exception of an extrapleural approach through a partial sternum splitting incision No death occurred All patients improved

*G R Wallgren*

G Petterson (Gothenburg Sweden) *Experience in reconstructive surgery for urinary incontinence in children*

H Sommerschild (Uppsala, Sweden) *Renal concentrating ability in children with chronic pyelonephritis*

Evaluation of $T_cH\,O_{max}$—maximal reabsorption of free water—during *mannitol* diuresis in dehydrated patients was performed to evaluate if this was a better test for the renal concentrating ability against estimation of maximum urinary specific gravity

T H O is calculated from the following equations

$$\textit{Osmolar clearance} = \frac{\text{Urine osmolality}}{\text{Serum osmolality}} \times \text{Urine volume}$$

T H O = Osmolar clearance + urine volume

Eleven patients received 10 % mannitol by intravenous infusion over a 2 hour period at a rate of 3 mOsm/kg/h after fasting for 12–14 hours Blood and urine samples were taken every 15 min

One control patient showed normal values

Four patients with isostenuria had definite *pathologic values for* $T\,H\,O_{max}$ Four patients with urinary specific gravity within normal range showed normal values for $T_cH\,O_{max}$ as well

In 2 patients with borderline urinary specific gravity T H O    was subnormal indicating decreased renal concentrating capacity

The method will not replace the estimation of urinary specific gravity but may be of value in selected cases where evaluation of the renal function is essential

A Flatmark & O Knutrud (Oslo Norway) *The significance of distal urethral stenosis in girls*

The incidence of distal urethral stenosis in girls reported in the American literature has been increasing It is, however difficult to pinpoint exactly what is meant by the term meatal stenosis Burrows (1965) said that it implied an individual with functional obstruction

This is hard to understand in the light of the pinpoint meatus which often is seen in boys with hypospadias but no sign of infection or dilatation of the upper urinary tract

From 1964 to 1967 the authors have diagnosed meatal stenosis in 57 girls with recurrent urinary infection They all had a round meatus with a diameter of 2–3 mm Their average was 7 ½ years

A bladder hypertension with an average *max detrusor value of 93 4 mm Hg* and a bladder volume of 188 ml were found A posterior meatotomy with dilatation was performed and postoperative cystometric studies were done six months and one year later The postoperative values show a slight but not significant decrease in the detrusor values (74 8 mm Hg) and a slight not significant, increase in the bladder capacities (210 ml) The accommodation curves show a tendency towards less tension postoperatively but not of the degree found by Gjertsen (1961) using the same cystometric method in prostatic patients

Only in 6 patients a normalisation of all the cystometric parameters were found

This investigation shows clearly that the diagnosis of urethral meatal stenosis in girls should be used with the strictest criteria

Johs S Rø D Knutrud & H Stormorken (Oslo Norway) *Reimplantation of ureter in bladder in children under antifibrinolytic treatment with tronexamic acid (AMCA)*

This operation is very seldom accompanied by generalized fibrinolysis or considerable blood loss the usual indications for antifibrinolytic treatment A minor blood loss in children however can make blood transfusions necessary with the danger of immediate and late complications

In a more heterogeneous series with the antireflux operation of Leadbetter Politano, blood

transfusions were given frequently to 54 out of 103 children It is therefore important to try to reduce the frequency of transfusions As some of the transfusions were unnecessary more appropriate indications would reduce such unnecessary transfusions

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*G R Wallgren*

## NEW BOOKS RECEIVED

H Sommerschild Sundby & P C Kreyberg *Prognosis in child psychiatry* 118 pp Universitetsforlaget Oslo 1968 Price not given

G H Valentine *Die Chromosomenstörungen Eine Einführung für Kliniker* 152 pp 74 figs Springer Verlag Berlin Heidelberg New York 1968 DM 14 80 US $3 70

Ch Varga *Handbook of pediatric medical emergencies* 4th ed 694 pp illus C V Mosby Co St Louis Missouri 1968 US $19 75

D H Shmerling H Berger & A Prader (eds) *Symposium on intestinal absorption and malabsorption* 215 pp illus S Karger Basel New York 1968 sFr/DM 50— US $12

C E Ford & H Harris (eds) *New aspects of human genetics* 117 pp British Medical Bulletin Vol 25 London 1969 £2

D I Williams (ed) *Paediatric urology* 385 pp Butterworth & Co London 1968 £8 10s

D Janz *Die Epilepsien Spezielle Pathologie und Therapie* 554 pp G Thieme Verlag Stuttgart 1969 DM 118—

J L Melnick (ed) *Progress in medical virology* 502 pp illus S Karger Basel New York 1969 sFr/DM 88— US $21 10

L I Gardner (ed) *Endocrine and genetic diseases of childhood* 1072 pp illus W B Saunders Co London Philadelphia 1969 £14

D B Jelliffe *Infant nutrition in the subtropics and tropics* 2nd ed World Health Organization Monograph Series No 29 Geneva 1968 US $9

H M van Praag (ed) *Brain damage by inborn errors of metabolism* 176 pp De Erven F Bohn N V Haarlem 1968 US $3 75

R A McCance & E M Widdowson (eds) *Calorie deficiencies and protein deficiencies* 386 pp illus J & A Churchill Ltd London 1968 80s

N S Scrimshaw C E Taylor & J E Gordon *Interactions of nutrition and infection* 329 pp World Health Organization Monograph Series No 57 Geneva 1968 US $9 00

G de Toni (ed) *Auxologia* Vol 2 *Auxologia postnatale fisiologica* 879 pp Edizioni Minerva Medica Torino 1968 Price not given

W Ehrengut *Allergie und Immunitätslage nach Vaccinia- und Variolainfektion* 85 pp Ferdinand Enke Verlag, Stuttgart 1968 DM 26—

H Stutte & H Harbauer (eds) *Concilium Paedopsychiatricum* Proc 3rd Europ Congr Paedopsychiatry 554 pp illus S Karger AG Basel New York 1968 sFr/DM 85—

J Mead *Helen's victory—the story of a chest illness* 80 pp illus Health Horizon Ltd London 1969 US $2 50

C E Alken V W Dix W E Goodwin H M Weyrauch & E Wildbolz (eds) *Encyclopedia of Urology Vol VII/1 Malformations* 479 pp 348 figs Springer Verlag Berlin Heidelberg New York 1968 DM 196— US $49 00

F Vasella (ed) *Aspekte der pädiatrischen Neurologie* 113 pp Pädiatrische Fortbildungskurse für die Praxis Vol 24 S Karger AG Basel New York 1968 sFr/DM 26—

P P Rickham & J H Johnston (eds) *Neonatal Surgery* 633 pp illus Butterworth & Co London 1969 £8 10s

D Lüders (ed) *Lehrbuch für Kinderkrankenschwestern Band II Das kranke Kind seine Pflege und Behandlung* 662 pp illus Ferdinand Enke Verlag Stuttgart 1969 DM 46—

## BOOK REVIEWS

H Schwarz *Herzchirurgie beim Säugling und Kleinkind* 158 pp illus Springer Verlag Berlin Heidelberg & New York 1968 DM 45—

The surgical treatment of congenital heart disease in infants and small children has not to any large extent been reviewed in the literature The need for such a review is to day very obvious

The author working at professor Senning's department in Zurich describes the congenital heart disorders and different surgical techniques He also presents the department's own experiences based on a large series of patients who were operated upon with good results

The operation methods correspond on the whole to those used in Sweden The reviewer feels however a little sceptical about the large number of banded pulmonary arteries (25 cases) in patients with interventricular septal defects In Sweden there has been less need of such an operation as the heart incompensation nearly always is relieved by medical treatment

The typographical arrangement makes the book

difficult to read The illustrations are of high quality especially those from the operations The book is warmly recommended It makes a valuable complement to the literature of heart surgery

*Sigrid Soderlund*

O Tonz *The congenital methemoglobinemias* Bibliotheca Haematologica No 28 146 pp ill S Karger Basel & New York 1968 sFr/DM 39

The authors describe two families with congenital methaemoglobinaemia In one of these the cause was an abnormal haemoglobin (Hb M ) Forty six members of the family were known to have been cyanosed since birth but were otherwise healthy The investigations mainly performed on blood from one of the members indicated that Hb M is identical to Hb M The inheritance was autosomal dominant

In the other family methaemoglobinaemia was caused by lack of erythrocyte NADH diaphorase Three homozygotes sixteen heterozygotes and eight normals were studied The enzymatic defect could be demonstrated both chemically and morphologically The homozygotes had been cyanosed since birth but were otherwise healthy

The greater part of this book is dedicated to a review of the literature and to descriptions of laboratory tests The book will be of help for the clinician who is performing an investigation of a case of congenital methaemoglobinaemia

*Lars Wranne*

G de Muralt (ed ) *Physiologie und Pathologie der Pubertat* Padiatrische Fortbildungskurse fur die Praxis Bd 3 II+173 pp 68 fig 26 tab S Karger AG Basel/New York 1968 sFr 39

These proceedings of the annual meeting 1967 of the Swiss paediatric society have been collected in a volume which is also no 23 in series of postgraduate courses in paediatrics published by S Karger in Basel Not less than fourteen authors all wellknown specialists in their respective fields have contributed and different aspects of puberty is dealt with Endocrine psychological social and legal problems are considered and two lectures are devoted to late or absent and precocious puberty The concluding remarks by A Prader emphasizes the very important fact that for the treatment of different disorders in adolescence it is not enou h to be familiar with the disease one must also understand the special somatic and psychological situation of the adolescent The volume is certainly a valuable contribution to a better understanding of the problems of puberty and adolescence even if one could have wished a more detailed discussion of some topics

*C G Bergstrand*

G A von Harnack (ed ) *Kinderheilkunde* 451 pp illus Springer Verlag Berlin Heidelberg & New York 1968 DM 38 —

This book gives in 450 pages a condensed but nevertheless a rather comprehensive survey of pediatric medicine The aim of the book is not to replace larger text books on pediatrics but to give an introduction to this field of medicine The book is systematically divided in chapters and subheadings and therefore easy to survey The illustrations—mostly drawings—are simple but most often very clearly arranged However as the book is intended to be a guide to pediatrics it would have been useful for some references to special literature to be given at the end of each chapter The book can be recommended as a text book to nurses training in pediatrics who prefer German to English language

*Tor Lindberg*

# ANNOUNCEMENTS

## THIRD INTERNATIONAL CONFERENCE ON CONGENITAL MALFORMATIONS

The third International Conference on congenital Malformations will be held in the Hague the Netherlands Sept 7–13 1969 Further information can be obtained from the conference secretariat c/o Holland Organizing Center 16 Lange Voorhout the Hague the Netherlands

*An International Symposium on Medical Problems of Adolescence* will be conducted in Athens Greece on September 26–27 1969 immediately preceding the 6th Middle Eastern Mediterranean Pediatric Congress

The Symposium will be conducted in English

Pannelists will be physicians and other who work with adolescents

Program Chairman is Professor Saul Blatman of New York and the whole Symposium is under the Chairmanship of Professor Spyros Doxiadis President of the Institute of Child Health

For further information please write to the Institute of Child Health Athens 608 Greece

*Acta Pædiat Scand 58 433–444 1969*

# FAMILIAL HYPOMAGNESEMIA

## *Biochemical histological and hereditary aspects studied in two brothers*

J H STRØMME R NESBAKKEN TRINE NORMANN F SKJØRTEN
D SKYBERG and B JOHANNESSEN

*From the Department of Clinical Chemistry Rikshospitalet the Institute of General and Experimental Pathology Rikshospitalet the Department of Pathology Ullevaal Hospital the Department of Pediatrics Rikshospitalet University of Oslo Oslo and Porsgrunn Lutherske Sykehus Porsgrunn Norway*

A case of neonatal hypomagnesemia due to a selective defect in the intestinal absorption of magnesium has recently been reported by us (22) The close similarity of the clinical picture and biochemical findings in our patient to three previously reported cases (4 18 20) suggests a new clinical entity On the basis of two additional cases we now designate this disease familial hypomagnesemia

The present report deals with clinical biochemical histological and electron microscopical data from two brothers In the older patient the diagnosis was made *post mortem* he died at 8 weeks of age having never received any magnesium therapy The younger brother who has daily magnesium as the only form of therapy has developed normally The familial occurrence of hypomagnesemia has not been reported previously

### *Case Report and General Studies*

*Case No 1* R B was male infant born on May 10th 1966 He was the first born of unrelated healthy parents in whom the electrolyte values are normal No cases of magnesium abnormality could be detected in the family The patient was born at term after an uneventful pregnancy and delivery the birth weight was 2 700 g and the length 49 5 cm

The infant diet consisted of breast milk for the first 10 days of life followed by diluted cows milk The baby was well until the 15th day of life when he began to have left sided seizures associated with involuntary eye movements upwards Three additional seizures of the same type occurred the following day and he was admitted to the hospital (Porsg Lutherske Sykehus)

On admission tetanic manifestations were served along with carpopedal spasms and positiv Chvostek and Trousseau signs The serum calcium was 3 5 mEq/1 and the phosphorus 3 1 mMol/ (Fig 1)

The patient received oral and intravenous calcium in high doses but the serum calcium level remaine low during the entire period of observation (Fig 1 The child experienced daily convulsions from th third to the seventh day after admission In additio to calcium Vitamin D and antiepileptics were ad ministered without any effect on his clinical co dition He deteriorated during the remainder of h hospitalization with continuous seizures during th last two days he died at age 50 days

No evaluation of the magnesium metabolism w carried out and no magnesium therapy was give A limited autopsy was performed the findings w be discussed below

*Case No 2* A B the brother of R B, is a ma infant born on August 16th 1967 The pregnan-, and delivery were uneventful and the birth weight was 3 560 g and the length was 51 cm His diet consisted of breast milk for the first few days of life followed by diluted cows milk and later on by evaporated milk

The baby was well until the 23rd day of life when he suffered a two-minute generalized seizure with involuntary eye movements upward and opisthotonus There was also some mild cyanosis There was no postictal state

Similar attacks occurred three and four days and he was admitted to the hospital (Porsgrunn Lutherske Sykehus) An identical seizure was seen shortly after admission Clinical examination revealed muscular hyperirritability a positive Chvostek sign and carpopedal spasm Laboratory evaluation showed a serum calcium of 3 8 mEq/1 serum phosphorus of

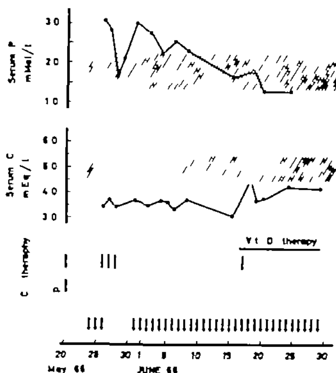


*Fig 1* Biochemical data and calcium therapy of Case No 1 during the age period 2 to 8 weeks Scratched areas indicate the normal range ∫ = Convulsions Vitamin D therapy is indicated by a horizontal line

2 8 mMol/1 and serum magnesium of 0 5 mEq/1 (Fig 2) The next day the patient received 2 mEq of magnesium chloride parenterally and was transferred to the Department of Pediatrics Rikshospitalet (University of Oslo)

Intravenous magnesium the only therapy given to this patient resulted in normal serum levels of phosphorus calcium and magnesium no further seizures were noted (Fig 2) After normalization of the electrolyte disturbances the parenteral dose of magnesium was varied in order to obtain information on the daily requirements It will be seen from Fig 2 that stable values for all electrolytes were obtained with a dosage in the range of 3 to 4 mEq/day given as three separate doses intramuscularly Since then the child has received magnesium lactate solution in doses of 10 to 20 mEq/day given with meals in six divided doses On this therapeutic regimen he has developed normally and has had no further seizures The serum values of magnesium have been stable in the subnormal range (1 1 to 1 4 mEq l) whereas the serum calcium and phosphorus have both been normal (4 6 to 5 7 mEq/1 and 1 50 to 1 65 mMol/1 respectively)

The magnesium supplementation was discontinued on one occasion in order to investigate his magnesium metabolism During this time a gradual fall in the serum magnesium occurred no marked changes were observed in the serum calcium or phosphorus

*Additional findings during therapy* The concentration of calcium and magnesium in the erythrocytes was normal (2 4 and 5 1 mEq/1 respectively) The concentration of calcium and magnesium in the cerebrospinal fluid was 2 3 and 2 1 mEq/1 (normal range for calcium 2 1-2 7 normal range for magnesium 2 4-3 0 mEq/1) the simultaneous serum values for calcium and magnesium were 4 7 and 1 4 mEq/1 respectively The protein content and the cell count of the cerebrospinal fluid were normal

Acid phosphatases in the serum (method Bessy *et al* (1) were found in the range 14 2 to 31 6 I U/1 in 8 blood samples drawn between the ages of one and six months The normal range for this age group is 17 6± 3 5 I U/1 (1 SD) (10) The tartarate inhibited fraction was found to be in the range of 1 1 to 4 4 I U/1 3 out of 5 examinations were above the normal range of 0 0 to 2 4 I U/(10) The increased levels of acid phosphatases are in agreement with our previous findings in a similar case (22) The alkaline phosphatase in the serum (method of Bessy *et al* (1) were found in the upper range of normal for this age group (79 to 138 I U/1)

Serum *GPT* was elevated in three samples (90 to 92 I U/1) *GOT LDH* and *CPK* were all normal

The prothrombin-proconvertin value as well as the thymol turbidity test were normal

The total serum proteins were somewhat low 5 5 to 6 1 g/100 ml and the electrophoretic separation revealed a low albumin fraction durin the first two

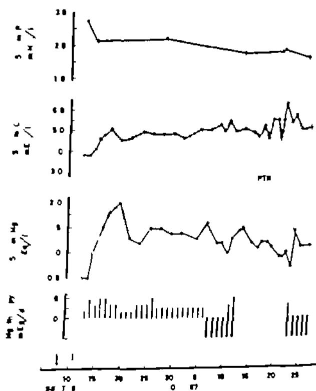


*Fig 2* Biochemical data and magnesium therapy of Case No 2 during the period 3 to 10 weeks of age The magnesium therapy is indicated by vertical columns oral therapy downwards intravenous therapy upwards from the zero line ∫ = Convulsions | = PTH Parathyroid hormone injections (1 ml × 2 in two days)

months of life and a low gamma globulin with a normal albumin fraction at an older age

The hemoglobin concentration was 10 8-11 5 g/100 ml The white cell count platelet count and differential count of the white blood cells were normal Examination of bone marrow showed normal erythropoiesis and myelopoiesis but an increased deposition of hemosiderin The serum iron concentration was 45 $\mu$g/100 ml and the total iron binding capacity was normal

The serum concentration of sodium potassium chloride urea creatinine and glucose was normal The acid base status (pH pCO2 and standard bicarbonate) of the blood was normal

The urinary excretion of aldosterone 17 OH and 17 O-corticosteroids was normal (3 $\mu$g/day 0 3 mg/day and 0 1 mg/day respectively) The urinary excretion of amino acids was within the normal range

Chromosome analyses of lymphocytes kindly performed by Dr C B van der Hagen at the Institute of Medical Genetics University of Oslo showed normal masculine karyotype

Electroencephalographic recordings were done on three occasions at the ages of 1 3 and 5 months The first two recordings showed a moderate degree of slow waves in the background activity whereas the last one was normal Electromyography revealed a normal pattern

X ray examination of the skeleton demonstrated normal bone structure and normal development of the ossification centres

## BIOCHEMICAL STUDIES

### *Procedures and Methods*

Radioactive Mg (specific activity 30 $\mu$Ci mg Mg) was obtained from Brookhaven National Laboratories Upton N Y Bovine parathyroid hormone was purchased from Abbott Laboratories N Chicago

Table 1 *Recovery (per cent of dose) of* $^{28}$*Mg in feces and urine upon its peroral administration*

$^{28}$MgCl was administered in doses of about 8 $\mu$Ci containing approximately 0 1 mEq of inactive magnesium The urine and feces were collected during 5 days i e until no significant radioactivity could be detected The net absorption (per cent of dose) represents the difference between the radioactivity given and that recovered in the feces The results obtained in Controls II and III have been previously reported (22)

| | Patient | Controls I | II | III | Mean of controls |
|---|---|---|---|---|---|
| Recovered in feces | 91 2 | 72 6 | 33 4 | 28 8 | 44 9 |
| Net absorption | 8 8 | 27 4 | 66 6 | 71 2 | 55 1 |
| Recovered in urine | 0 5 | 12 3 | 10 2 | | 11 3 |

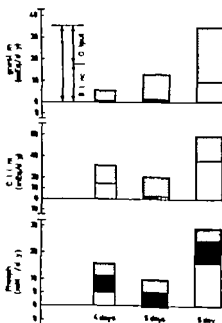


*Fig 3* Magnesium calcium and phosphorus balance data from Case No 2 The balance studies were done while the patient was on an ordinary diet during three separate periods during the first period of 4 days (columns to the left) no extra magnesium was given during the second (columns in the middle) and the third (columns to the right) periods of 5 days an extra supplementation of 9 and 24 mEq/day respectively was given The columns represent the average daily values obtained for the whole period The balance results are plotted essentially according to Moore & Ball (15)

III and magnesium lactate from British Drug Houses Ltd London

Studies with orally administered $^{28}$Mg were performed according to the procedure previously described (22) when the patient was 1 5 months old Balance studies of magnesium calcium and phosphorus were carried out for periods of 4 or 5 days at the ages of 2 3 and 5 ½ months During the first period no supplementation of magnesium was given (the medication had been withdrawn two days prior to the test) whereas during the last two periods 9 and 24 mEq of magnesium per day were given in addition to a normal dietary intake of magnesium Feces and urine were collected in colostomy bags during these periods Carmine red was given at the start and at the end of the test periods as a marker for intestinal transit time

Magnesium and calcium were determined by atomic absorption spectrometry essentially according to Willis (24) Phosphorus was determined by the automated procedure of Kessler & Wolfman (8) using the Technicon autoanalyzer Food and feces specimens were ashed and dissolved in a small volume of 2N $HNO_3$ and then diluted to appropriate volumes with distilled water prior to analysis The blood feces and urine specimens were assayed for γ activity with appropriate standards on a 3" thallium activated KJ crystal connected to a 400 channel pulse analyzer (Intertechnique Model SA 40) used in multiscaler mode Most of the results given in the Case Report and General Studies were obtained by routine analyses performed in our laboratory according to the conventional methods under daily control of standard and reference solutions

## *Results*

Results of the test with perorally administered Mg are given in Table 1 During the test the serum magnesium averaged 1 1 mEq/l It can be seen that about 91 • of the tracer was recovered in the feces during the 4 days which gives a net absorption of magnesium from the intestine of about 9 This degree of absorption is in the same order of magnitude as that found in the previously reported case (22) The net absorption found in these patients is markedly below that found in three control patients (age 6–12 months) who had demonstrated no abnormalities in their metabolism of magnesium and calcium Unfortunately one of the control patients (Control I Table 1) had diarrhoea during the test period which may be the reason for the somewhat higher recovery of magnesium in the feces of this patient

About 0 5 of the tracer dose was recovered in the urine during the test period (Table 1) This represents a urinary excretion of about 7 % of the absorbed radioactivity The corresponding values for the two control patients were 14 % and 46 •

Fig 3 shows the results of balance studies for magnesium, calcium and phosphorus during three periods During the first period no magnesium supplementation was given (dietary intake of 5 mEq/day) During the other periods a supplementation of 9 and 24 mEq/day in addition to the dietary intake was given this turned out to be 3 5 and 10 mEq/day respectively The differences in the dietary intake of the three periods are due partly to qualitative differences in the diet (diluted cow's milk in the first evaporated milk in the second) and partly due to quantitative differences The net absorption of magnesium as calculated from the first two balance studies was 9 of the dose which agrees well with the results obtained with ²⁸Mg whereas a much higher absorption was found in the last period The high absorption in the third period is most likely due to experimental error as the collection of the feces was incomplete during this time This result is, therefore omitted from further discussion

The magnesium balance was positive for the first two periods and increased from 0 4 mEq/day with no added magnesium to 1 1 mEq/day with magnesium supplementation Apparently an increase in the intestinal concentration of magnesium increases the amount absorbed The urinary excretion of magnesium during all the periods was low (0 15 0 06 and 0 08 mEq/day respectively) These findings exclude a pathological renal loss of magnesium as the cause of the hypomagnesemia Moreover the low urinary excretion even in the second period indicates that a net absorption of 1 1 mEq/day following a total oral load of 13 mEq magnesium per day is insufficient to meet the patient's demands In this connection it should be recalled that about 3 mEq/day of magnesium administered parenterally was necessary to maintain stable blood values (Fig 2)

Fig 3 also shows the balance of calcium and phosphorus in these periods It can be seen that a low net absorption of calcium was found when extra magnesium was given in the second period This was not reproduced in the third balance test, when an extra 24 mEq of magnesium a day was given in this period we found a net absorption of 59 Even taking the incomplete collection of feces into consideration (see above) this finding is consistent with the conclusion that about 40–50 of the calcium was absorbed Thus we have no in

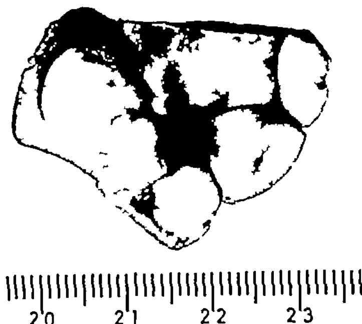



*Fig 4* Part of the liver from Case No 1 Note the coarsely nodular surface The scale is in cm

dication that magnesium decreases the absorption of calcium This is in accordance with the recent findings of Paunier *et al* (18) in a similar case

The above results indicate a defect in the intestinal absorption of magnesium The absorption of other compounds was therefore tested to see whether a generalized malabsorption syndrome could be found The fat content of the feces was examined on three occasions (sampling periods of 5 days) while the patient was on an ordinary diet The daily excretion was 0 6 0 9 and 1 7 g the fat constituting from 9 to 20 of the fecal dry weight Some 85 to 97 of the total fat was free fatty acids The xylose absorption was evaluated by determining its urinary excretion in 4 hours after an oral test load of 0 5 g per kg body weight Seventeen per cent of the test load was excreted which is in the lower range of normal The oral glucose tolerance test demonstrated a normal pattern of response of the blood glucose level These te ts rule out the existence of a generalized malabsorption state

The peripheral action of the parathyroid hormone following intramuscular administration was tested while the patient was hypomagne emic and slightly hypocalcemic The serum level of calcium increased whereas no definite change in the serum level of magnesium could be found (Fig 2)

## MORPHOLOGICAL STUDIES

### *Procedures and Methods*

In Case No 1 a limited autopsy was performed at the local hospital Specimens from the neck liver spleen and kidneys were examined histologically

In Case No 2 open biopsies were taken from the liver and abdominal muscle A biopsy from the jejunum was obtained by a Crosby capsule The tissues were examined under both light and electron microscopes

Light microscopy was performed on formalin fixed paraffin embedded material stained with hematoxylin-eosine-saffran In addition von Kossa s and Morin s stains for calcium and Turnbull s stain for iron were used

For electron microscopy the tissues were cut into small pieces fixed in 2 % ice cold glutaraldehyde (Union Carbide Corp New York N Y ) in M/15 phosphate buffer pH 7 4 for 2 hours, followed by ice cold 1% osmium tetroxide (Riedel de Haen AG Seelze Hannover Germany) in veronal acetate buffer

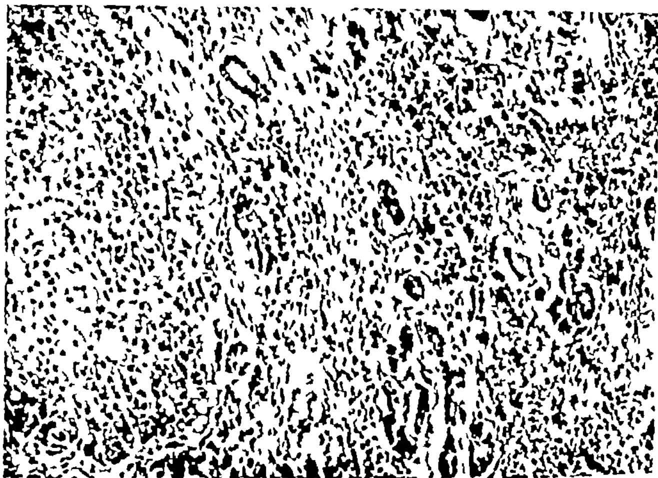


Fig 5 Histological section from liver of Case No 1 Note the large fibrous areas with scattered liver cells bile ducts and leucocytic infiltration To the left is better preserved liver parenchyma H+E Ini magn ×40

pH 7.4 containing 4.5 g glucose per 100 ml Tissues were dehydrated in alcohol and propylene oxide and embedded in Epon 812 Sections were cut on a LKB *Ultrotom III* Semithin sections for light microscopy orientation were stained with toluidine blue Ultra thin sections were stained with uranyl acetate and lead citrate and examined in a *Zeiss* EM 9A electron microscope

*Results*

*Case No 1* The liver on gross examination appeared coarsely nodular with yellowish scars (Fig 4) *Microscopic* examination re vealed small sharply demarcated areas with rel atively well preserved architecture alternating with large areas of complete collapse of the normal structure and extensive loss of paren chymal cells (Fig 5) In the latter areas only haphazardly distributed rosettes and small solid cords of liver cells were seen Few bi nucleated cells or mitoses were observed There was an abundance of connective tissue with moderate mononuclear infiltrate in the portal tracts around the central veins and dif fusely throughout the liver lobules In the sinus endothelium there were heavy deposits of he mosiderin (Fig 5) The blood vessels in the portal tracts were prominent and thick walled No bile duct proliferation was apparent Even the better preserved parts showed some in crease in connective tissue in the portal tracts and around the central veins The liver cells in these areas were vacuolated and small focal necroses with leucocyte infiltration were seen The Kupffer cells and to a lesser extent the parenchymal cells contained considerable amounts of hemosiderin

Histological examination of the removed parts of the neck revealed a normal thyroid gland a normal trachea, and a normal oeso-

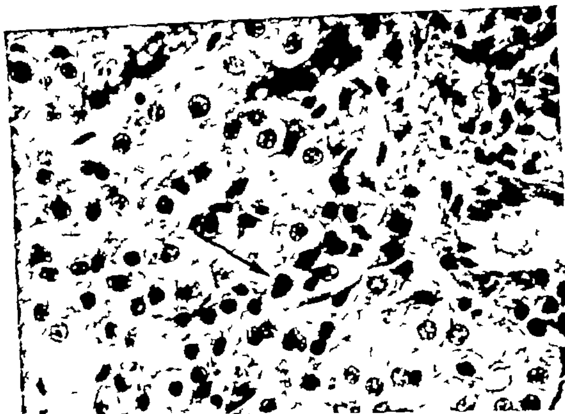


Fig 6 Histological section from the liver of Case No 2 Note extensive eosinophilic degeneration (arrow) H+E Init magn 40

phagus Parathyroid tissue could not be demonstrated but the material removed was not complete enough to exclude its existence The kidneys appeared normal Calcium deposits were searched for especially in the kidney (18) but could not be demonstrated The spleen showed congestion of the pulp and large amounts of hemosiderin

The histological picture thus showed a severe damage to the liver parenchyma with extensive fibrosis and marked deposition of iron in the reticuloendothelial system

*Case No 2* The liver appeared macroscopically normal and histological sections showed a normal lobular architecture There was a slight increase in connective tissue in the portal tracts and around the central veins and small collagenized septa were seen extending into the adjacent parenchyma In some areas the liver cells were arranged in pseudo-rosettes but few binucleated cells and no mitoses were seen Many liver cells showed eosinophilic degeneration (Fig 6) The Kupffer cells were prominent and filled with hemosiderin Some hemosiderin was found even in the parenchymal cells

Electron microscopy revealed liver cells of normal size The cell cords were partly replaced by small groups of liver cells surrounded by bundles of collagen which were limited by a basal membrane Scattered liver cells showed signs of necrosis

The rough and smooth endoplasmic reticulum was markedly dilated forming large intracytoplasmic lacunae which contained a flocculent material These lacunae occupied most of the cytoplasmic matrix Free and membrane bound ribosomes appeared to be reduced in number (Fig 7) The mitochondrial profiles were predominantly circular or oval, which

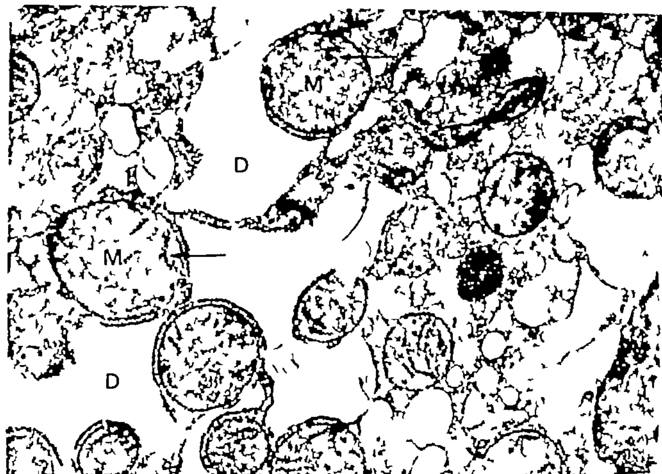



*Fig 7* Liver cell Dilated rough endoplasmic reticulum (*D*) Mitochondria (*M*) round and slightly swollen with dense bodies (*arrows*) in normal numbers Init magn × 6000

give them a swollen appearance The cristae seemed to be present in normal numbers The mitochondrial matrix was moderately electron dense and showed electron dense granules similar in number and size to the mitochondrial granules in normal liver (Fig 7) (9)

Glycogen was very sparse In some areas clusters of dense bodies containing ferritin like material were seen Scattered lipid droplets occurred in many liver cells

The nuclei and nucleoli were of normal size There was no margination of the nuclear chromatin in scattered, frankly necrotic liver cells

Sinusoids were lined by Kupffer cells which also showed dilation of the endoplasmic reticulum The space of Disse contained collagen fibrils in irregular distribution

The skeletal muscle appeared normal by light microscopy Structurally the muscle fibres also showed normal organization The mitochondria were of normal size and shape The matrix contained dense granules similar in number and appearance to those seen in the liver The sarcoplasmic reticulum was dilated and appeared empty whereas the transverse tubular system showed approximately normal caliber

The jejunum appeared normal by light microscopy Electron microscopically the villi showed an intact brush border with a normal cell coat covering (Fig 8) The crypts of Lieberkuhn showed normal organization The mitochondria were of normal size and shape The mitochondrial matrix showed scattered dense granules The rough and smooth endoplasmic reticulum was moderatly dilated and contained slightly electron dense flocculent material

In conclusion the main structural abnormality in this case was found in the liver Under the light microscope the changes appeared




*Fig. 8.* Jejunum. Brush border (*B*) with cell coat. Some dilation of the endoplasmatic reticulum in the basal part of the epithelium. Intercellular space (*ic*). Init. magn. 1800.

moderate with hepatocellular degeneration and necrosis, slight increase in connective tissue and increased hemosiderin deposits as the predominant features. Electron microscopy revealed in addition a markedly dilated endoplasmic reticulum particularly in the liver cells but also in the other organs examined. The mitochondria of the liver cells appeared swollen.

## DISCUSSION

The present paper deals with two brothers who at the ages of 15 and 23 days developed repeated tetanic convulsions. The tetany as well as the main biochemical derangements (hypomagnesemia, hypocalcemia and hyperphosphatemia) subsided when magnesium was given as the only form of therapy in Case No. 2. Magnesium was not given to the first patient; prolonged calcium medication failed to control his convulsions or to normalize his serum levels of calcium and phosphorus. Such a lack of response to calcium therapy has been a typical feature of the previously reported cases (22). Histological examination revealed liver damage as the most significant abnormality in both cases; this was severe in Case No. 1 and milder in Case No. 2. In spite of the fact that the magnesium metabolism was not evaluated in the first case, the similarity in age of onset, clinical manifestations, biochemical and histological findings makes it justifiable to conclude that the two brothers both suffered from the same disease.

An evaluation of magnesium metabolism in the second case revealed an abnormally low net intestinal absorption of the ion (Fig. 3, Table 1), whereas its renal handling appeared normal. No definite signs of generalized malabsorption were found either biochemically or

histologically Thus there seems to be a defect in the intestinal absorption of magnesium and the defect seems to be specific for this ion The same conclusions were reached in our previously reported case (22) and a low absorption of magnesium has also been found in similar cases reported by other authors (4 18) The absorption of calcium appears normal as judged from the balance studies the hypocalcemia in these patients is apparently secondary to the hypomagnesemia

Some of the data obtained in the second case have a bearing on the problem of the magnesium requirements in these patients It can be seen from Fig 2 that approximately 3 mEq/day of parenterally administered magnesium were necessary to keep the serum level stable Since some depletion of the body magnesium was most likely present at the time of the test this figure is probably somewhat above the requirements of a normal child of the same age Assuming a 10 % absorption of dietary magnesium as found in the patient an oral load of some 30 mEq/day should be necessary to meet the patients demand For practical reasons (tendency towards diarrhoea low solubility and bad taste of the magnesium salts) it has turned out to be difficult to exceed a daily dose of 20 mEq With a supplementation of this size the patient has been asymptomatic with normal serum calcium and phosphorus but with a persistently subnormal serum magnesium

A crucial question is how it is possible to combat the hypomagnesemia with orally administered magnesium in a case of deficient intestinal absorption Ross (19) has presented evidence that the absorption of magnesium at least in rats, depends on two separate mechanisms one is simple diffusion and the other is facilitated or active transport If we suppose that the latter mechanism is not operating in these patients their absorption will be directly proportional to the intraluminal concentration of magnesium and thus to the oral load of magnesium The balance studies show that the percentage absorption of magnesium at two different loads was nearly the same indicating a proportionality between oral load and absorption It is, therefore tempting to suggest that an active transport or facilitated transfer of magnesium normally takes place through the intestinal membrane and that this mechanism does not operate in our patient.

Although Vitamin D is known to increase the absorption of magnesium it has a greater effect on the absorption of calcium (5 6) In our case, it would be harmful to increase calcium absorption with Vitamin D because this would increase the load of calcium reaching the renal tubules Since there seems to be a competition between calcium and magnesium for tubular reabsorption (21) this would lead to an increased magnesium depletion Paunier et al (18) in a similar case found a decrease in the serum level of magnesium after administration of large doses of Vitamin D2 Furthermore nephrocalcinosis may threaten these patients (18) a fact which also argues against the use of this drug Therefore Vitamin D has been given only in the small prophylactic doses to patient No 2 and to the previously reported patient (22)

Following experimental hypomagnesemia in animals pathological changes in heart striated muscle kidney and vessel walls have been reported (7 11 13 16 17) Moore et al (16) and Larvor et al (11) have described hepatocellular degeneration and periportal fibrosis in calf liver These changes are comparable to those found in our patients Regarding the abnormal deposits of hemosiderin in the liver we cannot offer any definite explanation The most conspicuous change seen by electron microscope apart from liver cell necrosis was dilation of the endoplasmic reticulum In the liver this change was so severe that it indicated cellular damage (3) whereas in the other organs this change was less pronounced Signs of mitochondrial swelling were found in the liver cells Similar changes in the ultrastructure have been described in experimental magnesium deficiency in rats (7 17) We therefore assume that the histologic as well as the ultrastructural

changes found in our patients are mainly secondary to the hypomagnesemia. An increase in the amount of intra-mitochondrial dense bodies has also been frequently observed during experimental magnesium depletion (7). No such increase was detected in our patient, but it should be noted that the biopsies were taken during magnesium treatment.

Ultrastructural changes similar to those seen during the experimental magnesium deficiency have been produced by perfusion of cardiac muscle with high concentrations of calcium (12). Moreover, hypomagnesemia is known to promote an influx of calcium to the intracellular compartment (23). It is possible, therefore, that the structural changes seen in our patient are in fact due to a high intracellular calcium induced by the hypomagnesemia. As previously discussed (22), an accumulation of calcium intracellularly may also contribute to the *hypocalcemia* seen in our patients, and an efflux of calcium to the extracellular space may explain the rapid restoration of the serum calcium observed following the institution of magnesium therapy.

We have presented clinical, biochemical and histological evidence for a familial occurrence of neonatal hypomagnesemia which suggests a hereditary genesis of this disease. In this connection it is interesting that the parents of the patient reported by Friedman (4) were first cousins.

Six definite cases (4, 18, 20, 22) and one doubtful case (14) of familial hypomagnesemia have been published, all of whom were male infants. In addition, similar findings have recently been made in a female infant (2). Thus it seems that this is a rare disease. Nevertheless, a correct diagnosis is of the utmost importance, since therapy is lifesaving and simple to carry out.

## SUMMARY


Two brothers are reported who at the ages of two and three weeks respectively demonstrated generalized tetanic convulsions. In both cases hypocalcemia and hypophosphatemia were found. The serum magnesium of the elder brother was not determined, whereas the younger was found to have severe hypomagnesemia. Calcium administration had no effect on the condition of the elder one, who died at the age of 50 days. In the younger, magnesium alone led to a normalization of the clinical as well as the biochemical picture. On daily oral magnesium supplementation the child is healthy and is developing normally.

Data are presented which indicate that the hypomagnesemia is due to a selective defect in the absorption of this ion. The low serum calcium appears to be secondary to a magnesium deficiency.

Morphological findings in autopsy and biopsy material are presented, demonstrating histological and electron microscopical changes most marked in the liver.

On the basis of a familial occurrence of this disease, a hereditary genesis is considered likely.


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*Acta Pædiat Scand 58 445–448 1969*

# TRISOMY 17–18

## *A Study of Five Cases Three of Whom Were Associated with Oesophageal Atresia*

B G BENADY and R. J HARRIS

*From the Departments of Paediatrics and Pathology Southmead Hospital Bristol Great Britain*

The syndrome associated with trisomy of chromosomes 17–18 was first described in 1960 (3 8) and since then more than 180 cases have been recorded

Five cases of proved trisomy 17–18 have been born in Southmead Hospital since 1964 the clinical features of all these cases are summarised in Table 1 Three of these also had oesophageal atresia and tracheo-oesophageal fistula which has only rarely been described in this syndrome and are described more fully

## CASE REPORTS

*Case 1* D. N was a female infant born by caesarean section for foetal distress after a pregnancy lasting 41 ( weeks complicated by hydramnios in a gravida aged 26

Birth weight was 2500 g head circumference 34 7 cm, crown heel length 46 cm Regular respiration was not established for five minutes and much mucus was aspirated from the pharynx

Clinical examination revealed a large head with prominent occiput and patent metopic suture The external ears, which were low set were small and abnormal in appearance The palpebral fissures were small, wide-set, and had an anti mongoloid slant there was a depression between the nostrils at the tip of the nose The mouth was small and there was neck webbing.

The hands were clenched with the third and fourth fingers overlapping the index fingers and there were bilateral ulnar supernumerary digits The feet showed very wide 1st interdigital clefts There was a cardiac systolic murmur and gallop rhythm The skin was scaly in places, with excess lanugo

An oesophageal catheter could not be passed into the stomach so that a clinical diagnosis of oesophageal atresia was made In view of the presence of multiple abnormalities and the poor condition surgical correction was not considered feasible and the child died aged two days

Chromosome analysis from blood and skin showed trisomy 17–18 At *post-mortem* there was oesophageal atresia with a lower tracheo-oesophageal fistula, patchy atelectasis in the lungs, Fallot's tetralogy a right sided diaphragmatic hernia, and a Meckel's diverticulum. The central nervous system was normal apart from narrowing of the anterior third of the falx

*Case 4*

(Figs 1 3) A. C was a female infant born by forceps delivery to a gravida 5 para 2 aged 33 after a 36-week pregnancy complicated by gross hydramnios in the last few weeks There was no history of illness, drug ingestion or irradiation in pregnancy and no family history of congenital abnormalities

Birth weight was 1820 g, head circumference was 34 5 cm and crown heel length was 45 cm She required intermittent positive pressure ventilation before spontaneous respiration was established

On clinical examination the eyes were small, with slit like palpebral fissures and an anti mongoloid slant. The mouth was small and triangular in shape and the external ears low-set and ill formed, with the skin of the pinna continuous with that of the mastoid (Fig. 2) There was excess lanugo especially over the face and slight webbing of the neck. The nipples were displaced laterally There was generalised hypotonia, and all reflexes were absent.

The nails were hypoplastic the fingers were held in flexion, with the second and fifth fingers overriding the third and fourth (Fig. 3) The thumbs were small palmar creases and triradii appeared normal the heels were protuberant

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Table 1 *Summary of clinical post mortem findings in 5 cases of trisomy 17–18*

| Case Number | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Sex | ♀ | ♀ | ♂ | ♀ | ♀ |
| Gestation (Weeks) | 41½ | 40 | 38 | 36 | 38 |
| Hydramnios | + | + | − | + | + |
| Birth weight (Grams) | 2500 | 1840 | 1645 | 1820 | 1958 |
| Resuscitation difficulty | + | + | + | + | − |
| Age of death | 56 hr | 48 d | 7 d | 18 hr | 15 h |
| *Cardiovascular* | | | | | |
| V S D | + | + | − | + | + |
| Absent atrial septum | − | − | − | | + |
| Coarctation | − | − | − | − | + |
| Fallot s tetralogy | + | − | − | − | − |
| Pulm stenosis | + | − | + | − | − |
| Tricuspid atresia | − | + | − | − | − |
| P D A | − | + | + | − | + |
| Single umb artery | − | + | ? | | |
| *Gastro intestinal* | | | | | |
| Oesophageal atresia + tracheooesophageal fistula | + | − | − | + | + |
| Diaphragmatic hernia | + | − | | − | − |
| Meckel s Diverticulum | + | − | | | − |
| *Renal* | | | | | |
| Fused kidneys | − | − | | | + |
| Horseshoe kidney | − | + | | | − |
| Duplication | − | + | − | | |
| Hydronephrosis | − | − | − | | |
| *Craniofacial* | | | | | |
| Head circumference | 34 7 cm | 30 5 cm | 30 cm | 30 5 cm | ? |
| Hypertrichosis | − | + | | + | + |
| Neck webbing | + | + | | + | + |
| Narrow anti mongoloid papebral fissures | + | + | + | + | + |
| Coloboma of disc | ? | | ? | | ? |
| Small mouth | | | | | + |
| High arched palate | | + | | − | |
| Micrognathos | | + | + | | |
| Low set malformed ears | + | + | + | | |
| Dip between nostrils | + | | − | | |
| Abnormal falx | + | | | − | |
| *Limbs and trunk* | | | | | |
| Short sternum | | + | + | + | + |
| Wide spaced nipples | | + | | + | + |
| Flexion deformity of fingers | + | + + | + | + | + + |
| Small thumbs | | | | + | |
| Polydactyly (hands) | + | | | | − |
| Hypoplastic nails | | | | + | + |
| Syndactyly 2nd–3rd toes | − | | + | − | − |
| Wide 1st interdigital cleft of feet | + | + | − | | |
| Abnormally shaped soles | + | + | | + | + |
| Hypotonia | + | − | + | + | + + |

+ present − absent ? not recorded



*Fig 1* Case 4 Showing infant as a whole

brim An oesophageal tube was arrested at 10 cm from the lips

She developed severe respiratory distress and died at the age of 18 hours

At *post mortem* chromosome analysis from blood and skin showed trisomy 17–18 There was oesophageal atresia with a fistula from the distal end joining the trachea at the carina hypoplasia of the clavicles and sternum a ventricular septal defect fused kidneys lying at the right pelvis brim atelectasis with hyaline membrane formation and intraventricular haemorrhage

*Case 5*

M T was a female infant born by normal vertex delivery to a gravida 6 para 5 aged 45 after a 38 week pregnancy complicated by gross hydramnios in

the last few weeks There was no history of illness drug ingestion or irradiation in pregnancy and no family history of congenital abnormalities The birth weight was 1960 g

Regular respiration was established within 10 minutes but the baby remained cyanosed much mucus was aspirated from the pharynx

On clinical examination the eyes were small with narrow anti-mongoloid palpebral fissures The mandible was hypoplastic the external ears were ill formed with the skin of the pinnae continuous with that of the mastoids There was neck webbing most marked on the right side the sternum was short and the nipples were displaced laterally

The fingers were fixed in flexion with the index fingers overriding the others the toes were small and the heels protuberant

There was no murmur audible over the praecordium but the second heart sound was accentuated in the pulmonary area The upper abdomen could be seen to distend with each breath An oesophageal tube was arrested at 8 cm from the lips

Thoracotomy was undertaken at the age of 6 hours, confirming the clinical diagnosis of oesophageal atresia with a fistula between the lower end of the oesophagus and the bifurcation of the trachea and



Fig 2 Case 4 Ear—showing poorly formed ear with skin of pinna continuous with that of the mastoid Note also the lanugo of the face



Fig 3 Case 4 Hand—showing typical position of fingers in the syndrome Note also the hypoplastic nails

cervical oesophagostomy and a gastrostomy were performed but she died at the age of 15 hours following haemorrhage from the oesophagostomy

Chromosome analysis from blood and skin showed trisomy 17 18 *Post mortem* This confirmed the clinical and operative findings In addition there was an absent atrial septum a large ventricular septal defect and unilateral hydronephrosis There were only eleven ribs on each side

## DISCUSSION

The incidence of trisomy 17–18 has been reported as 0 23 per thousand live births (9) The incidence in babies born in this hospital (1964–1967) is 0 28 per thousand live births A search through the records of all babies born with congenital abnormalities during this period failed to reveal any further possible cases in which the diagnosis might have been missed therefore the above is probably a true figure for the incidence of trisomy 17–18

The findings in our five cases are similar to those previously reported (10) (Table 1) apart from the presence of oesophageal atresia and tracheo-oesophageal fistula in the three cases described above This combination has only

Table 1 *Summary of clinical post mortem findings in 5 cases of trisomy 17–18*

| Case Number | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Sex | ♀ | ♀ | o | ^ | ♀ |
| Gestation (Weeks) | 41½ | 40 | 38 | 36 | 38 |
| Hydramnios | + | + | | + | + |
| Birth weight (Grams) | 2500 | 1840 | 1645 | 1820 | 1958 |
| Resuscitation difficulty | + | + | + | + | – |
| Age of death | 56 hr | 48 d | 7 d | 18 hr | 15 h |
| *Cardiovascular* | | | | | |
| V S D | + | + | – | + | + |
| Absent atrial septum | – | – | – | | + |
| Coarctation | – | | – | – | + |
| Fallot s tetralogy | + | | – | – | – |
| Pulm stenosis | + | – | + | | – |
| Tricuspid atresia | – | + | | | – |
| P D A | – | + | + | – | + |
| Single umb artery | – | + | ? | | |
| *Gastro intestinal* | | | | | |
| Oesophageal atresia tracheooesophageal fistula | | – | – | + | + |
| Diaphragmatic hernia | + | – | | | |
| Meckel s Diverticulum | + | – | | | – |
| *Renal* | | | | | |
| Fused Kidneys | – | – | | | + |
| Horseshoe Kidney | – | + | | | |
| Duplication | | + | – | | |
| Hydronephrosis | | – | | | |
| *Craniofacial* | | | | | |
| Head circumference | 34 7 cm | 30 5 cm | 30 cm | 30 5 cm | ? |
| Hypertrichosis | – | | | + | |
| Neck webbing | + | | | + | |
| Narrow anti mongoloid papebral fissures | + | | | + | + |
| Coloboma of disc | ? | | ? | + | ? |
| Small mouth | | | | + | |
| High arched palate | | + | | | |
| Micrognathos | | | | | |
| Low set malformed ears | | | | | |
| Dip between nostrils | | | | | |
| Abnormal falx | + | | | – | |
| *Limbs and trunk* | | | | | |
| Short sternum | | + | + | + | + |
| Wide spaced nipples | | + | | + | |
| Flexion deformity of fingers | + | + + | | + | + + |
| Small thumbs | | | | + | |
| Polydactyly (hands) | + | | | | |
| Hypoplastic nails | | | | + | + |
| Syndactyly 2nd–3rd toes | – | – | + | – | |
| Wide 1st interdigital cleft of feet | + | + | | | |
| Abnormally shaped soles | + | + | – | + | + |
| Hypotonia | + | – | + | + | + + |

\+ present – absent ? not recorded



*Fig 1* Case 4 Showing infant as a whole

brim An oesophageal tube was arrested at 10 cm from the lips

She developed severe respiratory distress and died at the age of 18 hours

At *post mortem* chromosome analysis from blood and skin showed trisomy 17 18 There was oesophageal atresia with a fistula from the distal end, joining the trachea at the carina hypoplasia of the clavicles and sternum a ventricular septal defect fused kidneys lying at the right pelvis brim atelectasis with hyaline membrane formation and intraventricular haemorrhage

*Case 5*

M T was a female infant born by normal vertex delivery to a gravida 6 para 5 aged 45 after a 38 week pregnancy complicated by gross hydramnios in

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# ON THE DIAGNOSIS OF SYMPTOMATIC NEONATAL HYPOGLYCEMIA

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Hypoglycemia is a rather common condition during the first days of life (2) Paradoxically while some newborns seem to be quite unaffected by a low blood glucose level others may exhibit serious clinical signs such as apneic spells convulsions or acute circulatory failure (2 3 11 12 24)

The syndrome of symptomatic neonatal hypoglycemia is by no means a true clinical entity It may be transient persistent or have a tendency to recur (28) The condition may be associated with inborn errors of carbohydrate metabolism such as glycogen storage disease (19 23) It has been observed also in the offspring of diabetic mothers (20) in the so-called infant giant or pseudofoetopathia diabetica syndrome (10 11 22) in leucine sensitive type hypoglycemia (17) and in hyperplasia or adenoma of the islet cells of the pancreas (7 25) Symptomatic neonatal hypoglycemia is most commonly diagnosed in infants with a birth weight which is low in relation to the gestational age i e in newborns who are "small for gestational age" or small for dates The clinical features of this group were recently reviewed (3) Symptomatic neonatal hypoglycemia has been observed also in full term infants of normal birth weight with no apparent associated abnormal features

The diagnosis of symptomatic neonatal hypoglycemia is easily made when the clinical features are typical and overt However if the signs are vague or nonspecific such as high pitched or weak cry jitteriness refusal to take food limpness or cyanosis it may be difficult to evaluate their relation to a coexisting low blood glucose concentration Similarly in infants with vague signs it may be difficult to draw definite conclusions from the clinical response to intravenously administered glucose

In this communication the results of intravenous glucose tolerance tests in newborn infants with and without clinical signs of hypoglycemia will be reported Repeated tests were performed during the neonatal period The results have been correlated to the clinical features to the immediate clinical course and to the prognosis

## CLINICAL DATA

*General survey*

Eighteen newborn infants with hypoglycemia i e with blood glucose concentrations of less than 20 mg per 100 ml were studied There were 11 males and 7 females Further clinical data regarding gestational age weight and length at birth are given in Tables 1 and 2

*Pregnancy and delivery*

Five mothers had toxemia of pregnancy (Cases 2 5 6 8 and 12) One mother had gestational diabetes with glycosuria and decreased intravenous glucose tolerance (Case 17) There were 3 sets of twins (Cases 4

This work has been supported by a grant from AB Semper Fund for Nutritional Research

been reported on four previous occasions (1 6 11 12) One case with tracheo-oesophageal fistula without atresia has been described (5)

No case of trisomy 13–15 has yet been described with oesophageal atresia (10) but in trisomy 21 it is a well recognised malformation (7) Holder *et al* (4) found 28 mongols in a series of 1058 cases of oesophageal atresia and tracheo oesophageal fistula

Trisomic syndromes are associated with a spectrum of clinical anomalies most of which are not specific to any one trisomy, and oesophageal atresia must now be numbered among these Even an abnormality which is typical of a trisomic syndrome such as arrhinencephaly in trisomy 13–15 may occasionally be seen in trisomy 17–18 as reported by Butler *et al* (2) and as found in a further case of trisomy 17–18 born in this hospital since this paper was prepared Thus it is clear that the clinical abnormalities found cannot be due to the effect of specific genes or groups of genes on the extra chromosome and the causation of particular anomalies is unlikely to be elucidated until more delicate methods of analysis are devised

Other malformations particularly of the lower alimentary tract and the heart are so frequently found with oesophageal atresia that it has become standard teaching and practice to investigate the infant carefully for these before proceeding to surgery which is necessary as early as possible after birth It is also important to be aware of the association that has now been shown with multiple abnormalities due to autosomal trisomy of 17–18 chromosomes

## SUMMARY

Three cases of trisomy 17–18 associated with oesophageal atresia and tracheo-oesophageal fistula are described and the clinical features of two other cases of trisomy 17–18 are summarised It is concluded that the association between the two conditions is not so rare as previous reports suggest

## ACKNOWLEDGEMENT

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*Key words* Oesophageal atresia trisomy 17-18

Table 2 *Clinical data and glucose disappearence rates in Cases 13–18*

| Case no | Sex | Gestational age (weeks) | Birth weight (kg) | Length (cm) | Condition at birth | Presenting symptoms age (days) | Other neonatal complications | k value age (days) Before treatment | Treatment |
|---|---|---|---|---|---|---|---|---|---|
| 13 | ♀ | 36 5 | 1 90 | 45 | Good | Jitteriness (2) | Hypocalcemia | 1 2 (4) | Breast milk only |
| 14 | ♂ | 36 5 | 1 73 | 44 | Slight asphyxia | Jitteriness (1) | — | 0 95 (5) | Breast milk only |
| 15 | ♂ | 37 | 2 78 | 45 | Good | Jitteriness (1) | Hyperbilirubinemia | 0 53 (3) | Breast milk only |
| 16 | ♂ | 40 | 70 | 47 | Good | Jitteriness (2) | — | 0 47 (2) | Breast milk only |
| 17 | ♀ | 39 | 2 36 | 44 | Good | Jitteriness (5 hrs) | — | 0 66 (5 hrs) | Breast milk only |
| 18 | ♀ | 40 | 2 49 | 47 | Good | Jitteriness (3) | — | 0 94 (3) | Breast milk only |

6 and 13) Hypoglycemia was observed in each smal ler infant. In Case 6 the second twin was born mac erated and in Case 13 the first twin was severely asphyxiated and died within a few minutes after birth One mother had a history of 11 previous abortions (Case 2) Four infants were delivered by caesarean section (Cases 1 5 7 and 8) The remaining 14 in fants were delivered vaginally and Case 3 by for ceps

### *Clinical data in the neonatal period*

Twelve of the infants were small for their gesta tional age (Figs 1 ) Their birth weights and lengths were well below the normal for the period of gesta tion according to Swedish standards (5) Two infants were large for gestational age i e with birth weights exceeding the expected mean value by 1 (Case 9) and 1 5 (Case 11) standard deviations re pectively Case 11 had features of an infant giant" The infant born by a mother with gestational diabetes (Case 1 ) had clinical features typical of the offspring of diabetic mothers Three of the infants were of normal weight and length in relation to their gestational age and hac normal external features

All 18 infants developed clinical signs which wer suggestive of a diagnosis of symptomatic neonata hypoglycemia, such as convulsions cyanosis with ap neic spells bradycardia, circulatory failure limpnes and jitteriness within 72 hours after birth (Tables and 2) Since the blood glucose level was found t be below 20 mg per 100 ml a diagnosis of symp tomatic hypoglycemia was considered and a diag nostic and therapeutic intravenous glucose load wa given in a dose of 0 5 or 1 0 g per kg body weight I 12 of the infants (8 boys and 4 girls) there wer immediate signs of improvement The criteria for diagnosis of symptomatic neonatal hypoglycemia wer thus fulfilled in these babies In the remaining 6 in fants who were all small for gestational age an who had jitteriness as the only abnormal sign ther was no apparent response to the glucose load Suc no association between the clinical signs and the lo blood glucose level could be demonstrated in the babies they were classified as cases of asymptomat neonatal hypoglycemia

The 12 babies with a diagnosis of neonatal sym tomatic hypoglycemia constitute a heterogeneous gro from a clinical point of view One infant had featur of the infant giant syndrome one was the produ of a mother with gestational diabetes one was lar for gestational age without having any specific exte nal features three were of normal weight for gest tional age (2 male and 1 female infants) None them was sensitive to leucine

### *Treatment and course*

In the same infants who responded favourably to initial intravenous glucose load treatment with co tinuous glucose infusion was instituted In additio treatment with hydrocortisone or human growth ho mone was started in 9 of these infants (see Table Furthermore in two of the infants as an extra the

Table 1 *Clinical data and glucose disappearence rates ($k_G$) before and after treatment in Cases 1–12*

| Case no | Sex | Gestational age (weeks) | Birth weight (kg) | Length (cm) | Condition at birth | Presenting symptoms age (days) | Other neonatal complications | k value age (days) Before treatment | After treatment | Treatment |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ♂ | 35 | 1 58 | 43 | Good | Cyanosis Bradycardia Convulsions (3) | Hypocalcemia Hyperbilirubinemia | 2 3 (3) | See Fig 4 | Glucose i v HGH total 6 mg during 2 days |
| 2 | ♂ | 37 | 2 43 | 47 | Good | Cyanosis Convulsions (2) | Hypocalcemia | 3 1 (2) | See Fig 5 | Glucose i v HGH total 1 5 mg during 2 days |
| 3 | ♂ | 40 | 2 20 | 45 | Good | Bradycardia Convulsions (1) | — | 3 8 (2) | See Fig 6 | Glucose i v Hydrocortison total 190 mg during 6 days |
| 4 | ♂ | 40 | 2 13 | 46 | Moderate asphyxia | Cyanosis Convulsions (2) | — | 4 1 (2) | 1 75 (3) | Glucose i v Hydrocortison total 50 mg during 4 days |
| 5 | ♂ | 38 | 2 18 | 46 | Slight asphyxia | Convulsions (2) | — | 1 4 (1) | 1 5 (5) | Glucose i v Hydrocortison total 180 mg during 5 days |
| 6 | ♀ | 40 | 2 70 | 45 | Good | Jitteriness Cyanosis (1) | Hypocalcemia | 3 5 (1) | 2 1 (3) | Glucose i v |
| 7 | ♀ | 38 | 2 87 | 50 | Moderate asphyxia | Jitteriness (2) | — | 2 75 (2) | 3 2 (6) 1 9 (11) | Glucose i v |
| 8 | ♂ | 39 | 3 10 | 48 | Good | Jitteriness (1) | — | 3 5 (1) | See Fig 8 | Glucose i v Hydrocortison 235 mg during 16 days |
| 9 | ○ | 40 | 3 79 | 50 | Good | Bradycardia Cyanosis Convulsions (2) | — | 1 4 (?) | 1 5 (3) | Glucose i v HGH total 3 mg during 1½ days |
| 10 | ♂ | 40 | 3 36 | 52 | Good | Convulsions (2) | — | 3 5 (4) | See Fig 7 | Glucose i v HGH total 7 mg during 2 days |
| 11 | ♀ | 35 | 3 56 | 50 | Moderate asphyxia | Limpness Cyanosis (2) | — | 3 4 (2) | See Fig 9 | Glucose i v HGH total 2 mg during 2 days |
| 12 | ♂ | 36 | 3 38 | 51 | Good | Jitteriness (1) Cyanosis (3) | Hyperbilirubinemia | 3 6 (3) | | Glucose i v |

Table 2 *Clinical data and glucose disappearance rates in Cases 13 18*

| Case no | Sex | Gestational age (weeks) | Birth weight (kg) | Length (cm) | Condition at birth | Presenting symptoms age (days) | Other neonatal complications | K value age (days) Before treatment | Treatment |
|---|---|---|---|---|---|---|---|---|---|
| 13 | ♀ | 36 5 | 1 90 | 45 | Good | Jitteriness (2) | Hypocalcemia | 1 2 (4) | Breast milk only |
| 14 | ♂ | 36 5 | 1 73 | 44 | Slight asphyxia | Jitteriness (1) | — | 0 95 (5) | Breast milk only |
| 15 | ♂ | 37 | 78 | 45 | Good | Jitteriness (1) | Hyperbilirubinemia | 0 53 (3) | Breast milk only |
| 16 | ♂ | 40 | 70 | 47 | Good | Jitteriness ( ) | — | 0 47 (2) | Breast milk only |
| 17 | ? | 39 | 2 36 | 44 | Good | Jitteriness (5 hrs) | — | 0 66 (5 hrs) | Breast milk only |
| 18 | | 40 | 2 49 | 47 | Good | Jitteriness (3) | — | 0 94 (3) | Breast milk only |

6 and 13) Hypoglycemia was observed in each smaller infant. In Case 6 the second twin was born macerated and in Case 13 the first twin was severely asphyxiated and died within a few minutes after birth One mother had a history of 11 previous abortions (Case 2) Four infants were delivered by caesarean section (Cases 1 5 7 and 8) The remaining 14 infants were delivered vaginally and Case 3 by forceps

*Clinical data in the neonatal period*

Twelve of the infants were small for their gestational age (Figs 1 2) Their birth weights and lengths were well below the normal for the period of gestation according to Swedish standards (5) Two infants were large for gestational age i e with birth weights exceeding the expected mean value by 1 (Case 9) and 1 5 (Case 11) standard deviations respectively Case 11 had features of an infant giant" The infant born by a mother with gestational diabetes (Case 1 ) had clinical features typical of the offspring of diabetic mothers Three of the infants were of normal weight and length in relation to their gestational age and had normal external features

All 18 infants developed clinical signs which were suggestive of a diagnosis of symptomatic neonatal hypoglycemia, such as convulsions cyanosis with apneic spells bradycardia circulatory failure limpness and jitteriness within 72 hours after birth (Tables 1 and 2) Since the blood glucose level was found to be below 20 mg per 100 ml a diagnosis of symptomatic hypoglycemia was considered and a diagnostic and therapeutic intravenous glucose load was given in a dose of 0 5 or 1 0 g per kg body weight In 12 of the infants (8 boys and 4 girls) there were immediate signs of improvement The criteria for a diagnosis of symptomatic neonatal hypoglycemia were thus fulfilled in these babies In the remaining 6 infants who were all small for gestational age and who had jitteriness as the only abnormal sign there was no apparent response to the glucose load Since no association between the clinical signs and the low blood glucose level could be demonstrated in these babies they were classified as cases of asymptomatic neonatal hypoglycemia

The 12 babies with a diagnosis of neonatal symptomatic hypoglycemia constitute a heterogeneous group from a clinical point of view One infant had features of the infant giant syndrome one was the product of a mother with gestational diabetes one was large for gestational age without having any specific external features three were of normal weight for gestational age (2 male and 1 female infants) None of them was sensitive to leucine

*Treatment and course*

In the same infants who responded favourably to an initial intravenous glucose load treatment with continuous glucose infusion was instituted In addition treatment with hydrocortisone or human growth hormone was started in 9 of these infants (see Table 1) Furthermore in two of the infants as an extra thera

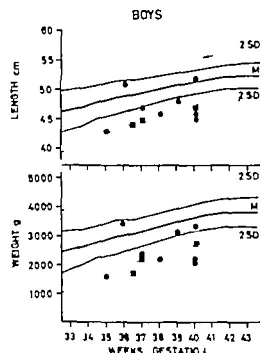



*Fig 1* Birth weights and lengths of boys with symptomatic (●) and asymptomatic (■) hypoglycemia plotted against weeks of gestation according to Swedish standards (5)

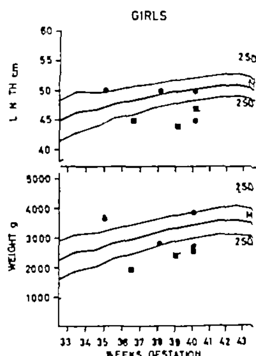



*Fig 2* Birth weights and lengths of girls with symptomatic (●) and asymptomatic (■) hypoglycemia plotted against weeks of gestation according to Swedish standards (5)

peutical trial infusions of a 10 per cent triglyceride emulsion (Intralipid® Vitrum) in a total dose of 0 5 g per kg body weight was given for 2 hours Treatment was discontinued slowly when the glucose level had returned to normal During the course of this study there were no further attacks of symptomatic hypoglycemia in any of the patients The 6 infants considered to have asymptomatic hypoglycemia were given only a high caloric supply using breast milk

*Follow up examination*

All patients with the exception of Case 9 were admitted for physical and neurological examination including electroencephalography at ages between 5 months and 2 years The developmental profile according to Buhler Hetzer was evaluated by an experienced psychologist Case 9 was examined in the out patient clinic

Table 3 *Clinical summary of maternal history in Cases 1–12*

| Case no | Previous pregnancies: Abortions | Previous pregnancies: Living children | Age | Current gestation | Delivery |
|---|---|---|---|---|---|
| 1 | 1 | 0 | 36 | Normal | Caesarean section |
| 2 | 11 | 0 | 36 | Toxemia | Vaginally |
| 3 | 0 | 0 | 20 | Normal | Vaginally Low forceps |
| 4 | 0 | 0 | 39 | Normal | Vaginally Second of twins First twin unaffected male b w 2 64 kg |
| 5 | 0 | 0 | 22 | Toxemia | Caesarean section |
| 6 | 0 | 0 | 25 | Toxemia | Vaginally First of twins Second twin female born macerated |
| 7 | 0 | 0 | 22 | Hypertonia | Caesarean section |
| 8 | 0 | 0 | 24 | Toxemia | Caesarean section |
| 9 | 0 | 3 | 32 | Normal | Vaginally |
| 10 | 0 | 1 | 36 | Normal | Vaginally |
| 11 | 1 | 1 | 28 | Normal | Vaginally |
| 12 | 0 | 2 | 35 | Glucosuria i v glucose tolerance test *k*$_G$ 1 0 Toxemia | Vaginally |

Table 4 *Clinical summary of maternal history in Cases 13–18*

| Case no | Previous pregnancies | | Age | Current gestation | Delivery |
|---|---|---|---|---|---|
| | Abortions | Living children | | | |
| 13 | 0 | 4 | 41 | Proteinuria | Vaginally Second of twins First twin b w 2 9 kg Apgar 0 died at birth |
| 14 | 0 | 5 | 40 | Normal | Vaginally |
| 15 | 1 | 0 | 24 | Pyelonephritis | Vaginally |
| 16 | 0 | 0 | 24 | Normal | Vaginally |
| 17 | 0 | 0 | 18 | Normal | Vaginally |
| 18 | 0 | 0 | 21 | Normal | Vaginally |

The results of the follow up examinations are summarized in Tables 5 and 6 Among the 12 patients with a diagnosis of symptomatic neonatal hypoglycaemia were found to have serious cerebral damage with microcephaly and gross atrophy of the brain as revealed from pneumoencephalograms (Cases 10 and 11) One was the baby with features of the infant giant syndrome The other patient was of normal birth weight in relation to gestational age A border line also for the developmental quotient and slight muscular hypertonicity was found in one infant (Case 4) who was small for gestational age at birth The examination revealed normal findings in the remaining 9 infants although the clinical signs of hypoglycaemia in the neonatal period had been very serious in some of them (see Table 1) All 6 patients with asymptomatic neonatal hypoglycaemia were completely normal at follow up examination

## BIOCHEMICAL STUDIES

### *Chemical methods*

Blood glucose concentrations were determined by means of a glucose oxidase method The blood samples were deproteinized with perchloric acid (0 3 *M*) and buffered to pH 2 7 with glycine (commercial method according to AB Kabi Stockholm) (Cases 1–12) or with sodium hydroxide and zinc sulphate (18) (Cases 13–18) Plasma free fatty acids (FFA) were determined by a modification of the method of Dole (26) and glycerol by Wieland s method (27)

### *Intravenous glucose tolerance*

After blood samples for determination of the pretest glucose levels a 25–30 glucose solution was rapidly injected intravenously in a dose of 0 5 to 1 0 g per kg body weight Capillary blood samples were then taken at intervals of 5 to 10 minutes for a period of one hour for glucose determinations The values were plotted against time on a semilogarithmic paper and the percentage disappearance per minute

Table 5 *Clinical summary of follow up examinations*

| Case no | Age at follow up years | Neurological findings | Electro-encephalo-graphy | Developmental quotient (Buhler Hetzer) | $k_g$ |
|---|---|---|---|---|---|
| 1 | 1 11/12 | Normal | Normal | 100 | 2 2 |
| 2 | 1 4/12 | Normal | Normal | 170 | 2 1 |
| 3 | 8 12 | Normal | Normal | 125 | 5 8 |
| 4 | 3/12 | Increased muscular tone | Normal | 80 | — |
| 5 | 1 7/12 | Normal | Normal | 96 | — |
| 6 | 5/1 | Normal | Normal | 93 | — |
| 7 | 1 4/12 | Normal | Normal | 131 | — |
| 8 | 5 1 | Normal | Normal | 100 | — |
| 9 | 9/12 | Normal | — | — | — |
| 10 | 2 | Brain atrophy | Abnormal | 35 | 3 0 |
| 11 | 1 11/12 | Brain atrophy | Abnormal | 30 | 3 9 |
| 12 | 8/1 | Normal | Normal | 100 | — |

Table 6 *Clinical summary of follow up examinations*

| Case no | Age at follow up years | Neurological findings | Electro encephalo graphy | Developmental quotient (Buhler Hetzer) | $k_a$ |
|---|---|---|---|---|---|
| 13 | 8/12 | Normal | Normal | 109 | 2 0 |
| 14 | 1 | Normal | Normal | 118 | 5 4 |
| 15 | 1 | Normal | Normal | 104 | — |
| 16 | 1 | Normal | Normal | 133 | — |
| 17 | 10/12 | Normal | Normal | 113 | — |
| 18 | 10/12 | Normal | Normal | 104 | — |

expressed as the $k_G$ value was calculated (15) In 11 out of the 12 infants with a clinical diagnosis of symptomatic neonatal hypoglycemia the $k_G$ values were estimated repeatedly during the immediate course In some of the patients the $k_G$ values were also estimated at follow up examinations

*Results*

In all patients the disappearance rate of intravenously administered glucose was studied in relation to the clinical response as a diagnostic criterion The results expressed as the $k_G$ values are given in Tables 1 and 2 and in Fig 3 Fig 3 also gives the mean $k_G$ values for normal full term infants during the first week of life (6) The values were higher in those infants who fulfill the criteria for symptomatic neonatal hypoglycemia than they were in those considered to have asymptomatic hypoglycemia In 10 out of the 12 infants with symptomatic hypoglycemia the $k_G$ value exceeded +2 SD in one baby (Case 5) the value was just above +1 SD and in another (Case 9) just below +1 SD

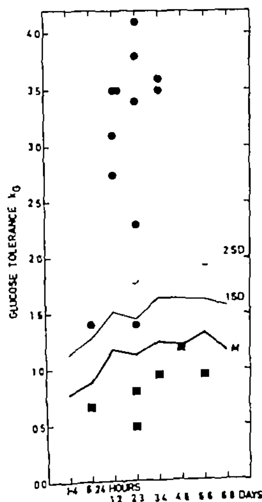



*Fig 3* Initial glucose tolerance ($k_G$) in infants with symptomatic (●) and asymptomatic (■) hypoglycemia plotted against age The mean $k_G$ value (M)+1 and 2 SD for normal full term infants during the f week of life are given for comparison (6)

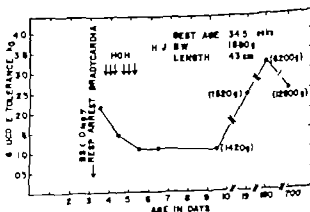



*Fig 4 Case 1* Changes in glucose tolerance ($k_G$) during the course of symptomatic neonatal hypoglycemia Classification infant with a birth weight which is low in relation to gestational age Human growth hormone is administered as indicated by the arrows weight in g is given within parenthesis

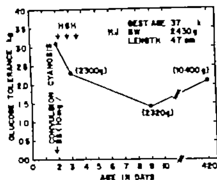


*Fig 5 Case 2* Changes in glucose tolerance (k ) during the course of symptomatic neonatal hypoglycemia Classification infant with a birth weight which is low in relation to gestational age Human growth hormone (HGH) was given as indicated by the arrows The body weight in g is given within parenthesis

The changes of the $k_G$ values during the course of symptomatic neonatal hypoglycemia are illustrated in Figs 4–9 In the infants small for gestational age there was a rapid drop of the valu s after the institution of treatment with human growth hormone (Figs 4–5) or with hydrocortisone (Fig 6) The same response occurred in the normal weight term infants (Figs 7 and 8) In one infant there was a transi nt

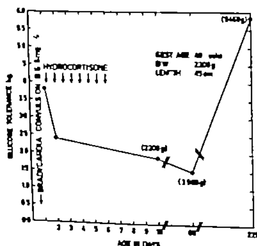


*Fig 6 Case 3* Changes in glucose tolerance ($k_G$) during the course of symptomatic neonatal hypoglycemia. Classification infant with a birth weight which is low in relation to gestational age Hydrocortisone was given as indicated by the arrows The body weight in g is given within parenthesis

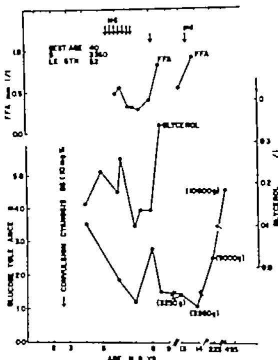


*Fig 7 Case 10* Changes in glucose tolerance (k ) and the plasma concentrations of free fatty acids (FFA) and glycerol during the course of symptomatic neonatal hypoglycemia Classification full term infant of normal birth weight with no apparent associated abnormal features Human growth hormone (HGH) and Intralipid® (triglyceride emulsion) were given as indicated by the arrows The body weight in g is given within parenthesis

rise of the $k_G$ value when treatment with human growth hormone had been discontinued (Fig 7) Also in the baby with infant giant syndrome the $k_G$ value dropped after treatment with human growth hormone (Fig 9)

In two of the patients with symptomatic neonatal hypoglycemia the plasma concentrations of FFA were followed concomitantly with the $k_G$ values In the patient who was full term and of normal birth weight (Case 10) the concentration of FFA was low before treatment and dropped even further after administration of human growth hormone (Fig 7) In the patient with infant giant syndrome (Case 11) the pre treatment concentration of FFA was high about 1 5 mmol per l (Fig 9) Treatment with human growth hormone again led to a significant drop

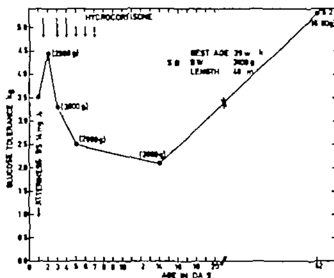



*Fig 8 Case 8* Changes in glucose tolerance ($k_G$) during the course of symptomatic neonatal hypoglycemia Classification full term infant of normal birth weight with no apparent associated abnormal features Hydrocortisone was given in decreasing doses as indicated by the arrows The body weight in g is given within parenthesis

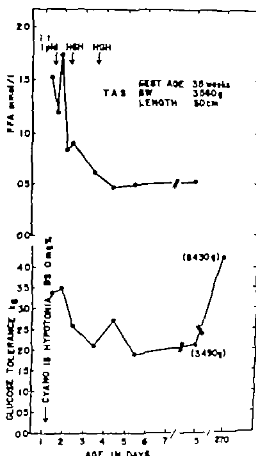



*Fig 9 Case 11* Changes in glucose tolerance ($k_G$) and the plasma concentration of free fatty acids (FFA) during the course of symptomatic neonatal hypoglycemia Classification infant giant syndrome Intralipid® (triglyceride emulsion) and human growth hormone (HGH) were given as indicated by the arrows The body weight in g is given within parenthesis

of the FFA-level The plasma concentration of glycerol followed a similar course to that of the FFA (Fig 7) Following the administration of Intralipid® (Figs 7 and 9) there was an increase of the concentration of FFA in plasma At the same time there was also a rise of the plasma glycerol concentration (Fig 7) There was no consistent relation between the $k_G$-value and the plasma concentration of FFA in Cases 10 and 11 in whom a number of simultaneous determinations were performed In one infant the infusion of Intralipid® was associated with a significant drop of the $k_G$-value from 2 8 to 1 4 (Fig 7) but in the other infant the $k_G$-value remained unchanged (Fig 9)

## DISCUSSION

From the clinical point of view the most important question is that of the relation of cerebral damage which appears to be a frequent consequence of symptomatic neonatal hypoglycemia (1, 3 13 22) Since brain damage as a result of hypoglycemia is thought to be preventable by early proper treatment it is urgent to find a method of establishing when hypoglycemic babies are at risk of developing this severe complication

There is general agreement that newborn infants can appear to be normal and remain so although the blood glucose concentration is extremely low but it is by no means settled that asymptomatic hypoglycemia is without danger to a newborn baby According the Shelley & Neligan (24) asymptomatic hypoglycemia is unlikely to cause cerebral damage yet, there are opposing reports (4) While our material is not complete enough to permit any conclusions regarding the long term prognosis in asymptomatic neonatal hypoglycemia, the results would tend to indicate that the condition does not cause cerebral damage by itself

Why some newborn infants seem to tolerate

very low blood glucose concentrations while others may develop serious signs such as convulsions or acute circulatory failure in association with hypoglycemia also remains unclear. A long standing marked hypoglycemia due to a rapid peripheral utilization of glucose may reduce the nutritional flow to a level critical for the central nervous system. The clearance rate of intravenously administered glucose is slow in those infants who are born at term of normal weight (6), in those who are small for gestational age and in those whose weights are appropriate for gestational age but born prematurely (9). The intravenous glucose tolerance increases during the first weeks of life, i.e. the $k_e$ value becomes higher (Fig. 3). In this study the removal rate of intravenously administered glucose was significantly increased in 10 out of 12 cases of symptomatic neonatal hypoglycemia before treatment as compared to normal newborns of corresponding ages. In the 6 infants in whom a diagnosis of asymptomatic hypoglycemia had been based on their response to the initial glucose injection, the $k_0$ values were found to be within the normal range. The finding of normal $k_e$ values in infants with asymptomatic hypoglycemia and of faster disappearance rates in infants with symptomatic neonatal hypoglycemia probably is of pathogenetic significance. Such a view finds support from the observation that the $k_0$ values declined concomitantly to a normalization of blood glucose levels after treatment with hydrocortisone or human growth hormone. A high $k_c$ value may indicate that glucose is peripherally utilized at such a rapid rate as to critically reduce the amount of glucose available to the central nervous system.

The cause of the rapid clearance rate of intravenously administered glucose in the babies with a diagnosis of symptomatic neonatal hypoglycemia is speculative since the factors which influence the removal rate are complex and incompletely understood. In the baby of a mother with gestational diabetes the rapid clearance of intravenously administered glucose was very likely due to an excessive and prompt release of insulin (16). Whether abnormally high insulin secretion was responsible for the development of hypoglycemia as well as for the high $k_0$ values in our other cases cannot be decided since no determinations of plasma insulin were performed. However, as compared to asymptomatic controls, relatively high fasting insulin levels at low blood glucose concentrations have been observed in cases of symptomatic neonatal hypoglycemia (2).

It is well recognized that hydrocortisone is effective in achieving normoglycemia in cases of symptomatic neonatal hypoglycemia (3). In those of our patients who were treated with hydrocortisone, intravenously administered glucose was cleared at a slower rate concomitantly to a normalization of the blood glucose level. Thus, the changes of the $k_c$ value were found to be a sensitive guide of the effectiveness of the treatment. The beneficial effect of hydrocortisone in symptomatic neonatal hypoglycemia may be due to an enhancement of gluconeogenesis and/or by modifying the response of peripheral tissues to insulin. However, the use of corticosteroids must be considered hazardous treatment in the newborn period. Cases where the use of steroids is likely to have caused transient diabetes mellitus have been reported (8).

Based on the observation that some of the infants with symptomatic hypoglycemia had low plasma concentrations of FFA, human growth hormone was administered in order to increase lipolysis. A redistribution of FFA from fat depots to other tissues would conserve available glucose for the central nervous system. Normoglycemia and normalization of the $k_0$ value was achieved, although a further decline of the plasma concentration of FFA was found in two cases. This decrease in plasma level of FFA was unexpected since human growth hormone normally causes an increase in FFA (14, 21). The simultaneous administration of glucose might have suppressed the FFA level (14, 21). However, in one patient the plasma FFA concentration was elevated by intravenous administration of Intralipid®. This was associated with a drop

of the $k_G$-value Whether this drop is explained by an increased utilization of exogenous fat remains speculative

Our findings seem to have some direct clinical implications and applications An estimation of the disappearance rate of intravenously administered glucose primarily given as a diagnostic test may differentiate between symptomatic and asymptomatic hypoglycemia If in a doubtful case the $k_G$-value is normal the likely diagnosis is asymptomatic hypoglycemia A high caloric intake will probably prevent the development of *symptomatic hypoglycemia* An abnormally high $k_G$ value indicates that additional therapy is needed Repeated determinations of the $k_G$ value seems to be a sensitive guide as to the effect of treatment


## SUMMARY

Eighteen newborn infants 11 males and 7 females with hypoglycemia i e with blood glucose concentration of less than 20 mg per 100 ml were studied Twelve infants were diagnosed as cases of symptomatic and 6 as cases of *asymptomatic hypoglycemia* All *infants* with asymptomatic hypoglycemia were small for gestational age whereas the symptomatic group was heterogenous from a clinical point of view

The disappearance rate of intravenously administered glucose ($k_G$ value) was studied before any treatment was started The $k_G$-values were high in all infants with symptomatic hypoglycemia in 10 exceeding +2 S D for normal infants of the same age The symptomatic infants were treated with hydrocortisone or human growth hormone in addition to continuous glucose infusion After the institution of therapy there was a rapid normalization of the $k_G$ values The 6 infants with asymptomatic hypoglycemia had normal $k_G$ values They were only given a high caloric supply by means of breast milk In two infants with symptomatic hypoglycemia repeated simultaneous determinations of $k_G$ values and the plasma concentration of free fatty acids revealed no relationship

At follow up examinations at ages from 5 months to 2 years 2 out of the 12 infants in the symptomatic group were found to have severe cerebral damage All infants with asymptomatic hypoglycemia were completely normal

The findings seem to have some clinical applications Determination of the disappearance rate of intravenously administered glucose primarily given as a diagnostic test may differentiate between symptomatic and asymptomatic hypoglycemia If in a doubtful case the $k_G$ value is normal the likely diagnosis is asymptomatic hypoglycemia In cases of symptomatic *neonatal hypoglycemia* repeated *determinations* of the $k_G$-value may provide a sensitive guide as to the effect of treatment



## ACKNOWLEDGEMENT

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Since this paper was submitted one of the patients with symptomatic neonatal hypoglycemia (Case 5) has had another episode of hypoglycemia with convulsions and a blood glucose level of 10 mg per 100 ml at an age of 1 year and 11 months Thus there is evidence of a persistent abnormality of carbohydrate metabolism in this particular patient

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*Key words* Newborn hypoglycemia symptomatic hypoglycemia asymptomatic hypoglycemia glucose tolerance glucose clearance glucose homeostasis cerebral damage

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# DIAGNOSIS OF SEVERE CONGENITAL HEART DISEASE IN INFANTS

L. BJÖRK, M. MICHAËLSSON and H. WENDEL

*From the Departments of Diagnostic Radiology and Pediatrics, University Hospital, Uppsala, Sweden*

New palliative methods for treatment suitable even for small infants have focused the interest on the detection and diagnosis of correctible congenital cardiac defects in early infancy.

The problems and difficulties in clinical roentgenologic and angiocardiographic diagnosis in children in early infancy are considerable and differ from those in older children and in adults. In the following our experiences in 103 consecutive cases with severe symptoms in infants under the age of one year are reported and an attempt at determining the value of different diagnostic methods used in these patients is made.

## MATERIAL

The material consists of 103 consecutive patients with symptoms of severe cardiac disease under one year of age referred to our hospital from September 1965 until the end of December 1967.

The principles for selection of patients being referred to us may have differed slightly but generally all patients under one year of age with a condition indicating severe cardiac disease have been referred for evaluation from a region of Sweden with a population of 1.9 million. Criteria for the severity of the cardiac disease were that the cases surviving were either operated or required a period exceeding one month of hospitalization with treatment of cardiac failure.

Sixty one of the patients were boys and 42 were girls. In Table 1 the patients are grouped according to diagnosis. As seen, only half of the patients are alive and one third have been operated on. Cardiac catheterization was performed in 78 patients and angiocardiography in 71. Twenty five patients were in such a bad condition that they died before cardiac catheterization was performed but they are included in this study since it was considered to be of value to present the findings and diagnosis in these cases as well. Four patients died before a more detailed clinical examination had been performed. These patients are included in Table 1 and they will not be included in the following presentation. The diagnoses in these four cases were "isolated" transposition of the great arteries, isolated pulmonary artery atresia, hypoplastic left heart syndrome and total anomalous pulmonary venous return. In 34 cases the diagnosis was verified at operation and in 49 at autopsy. One of us (M. M.) took part in the postmortem examination of all these 49 cases. In two other cases the autopsy of the heart (performed in other hospitals) did not disclose the exact nature of the cardiac malformations. In the remaining 18 cases the clinical course and the findings at follow up examinations are compatible with the diagnosis made at the initial investigation. The shortest follow up time in this group was one year.

## METHODS

*The clinical examination* included phonocardiography in all cases. A calibrated phonocardiogram was recorded by means of a six-channel ECG direct writing apparatus (Mingograph 81, Elema Company) with a phonopreamplifier. Filters with nominal frequencies of 25, 50, 100 and 200 c/s and an auditory amplification (5) were used. The paper speed was 100 mm/s.

*The electrocardiogram* was recorded with the same apparatus. Leads I, II, III, aVR, aVL, aVF, V, V, V, V, V and V were obtained. The criteria of Ritter *et al.* (7) for hypertrophy were followed.

*Conventional roentgen examination.* In all patients conventional radiograms of the chest including frontal, lateral and right and left anterior oblique projections of the heart were obtained. The volume of the heart was calculated from the standard radiograms according to the method described by Jonsell (4). As a referecence for normal cardiac size the figures given by Ghaffarpour *et al.* (3) were used.

*Heart catheterization* was made from the leg in all 78 patients. Vena saphena magna or vena femoralis distally to the entrance of vena femoralis profunda was used and in five cases where an atrial septostomy was performed the common femoral vein was used. Catheters number 5 or 6 F were used

Table 1. *Material of severe congenital heart disease in infants listed according to diagnosis*

| Diagnosis | No. of patients | Dead | Operated |
|---|---|---|---|
| Transposition of the great arteries, isolated | 10 | 3 | 9 |
| Transposition of the great arteries with other malformations | 7 | 4 | 2 |
| Pulmonary atresia, isolated | 4 | 4 | 1 |
| Pulmonary atresia with other malformations | 9 | 6 | 1 |
| Hypoplastic left heart syndrome | 13 | 11 | |
| Tricuspidal atresia | 9 | 6 | 1 |
| Ventricular septal defect | 8 | 1 | |
| Persistent ductus arteriosus | 9 | 1 | 8 |
| Fallot's anomaly | 5 | 3 | 4 |
| Common atrioventricular canal | 5 | 2 | |
| Pulmonary stenosis (valvular) | 4 | | 4 |
| Aortic stenosis | 2 | | 1 |
| Total anomalous pulmonary venous return | 2 | 2 | |
| Vascular ring | 2 | | 2 |
| Anomaly of coronary arteries | 1 | 1 | 1 |
| Other diagnoses | 11 | 5 | |
| No definite diagnosis | 2 | 2 | |
| Total | 103 | 51 | 34 |

and in the septostomy cases the Rashkind catheter number 6.5 F (6) was used. In 56 out of the 78 patients where a right heart catheterization was performed the left heart was reached through an atrial septal defect or through foramen ovale.

*Angiocardiography* was performed in 71 patients. Whenever possible the principles of selective angiocardiography have been followed. In a few cases this was impossible and the injections were then made in the right atrium and in one case in the abnormal inferior vena cava. 0.5–2.0 ml of contrast medium per kg of body weight was used for each injection. The total dose of contrast medium varied between 0.5 and 2.0 ml per kg of body weight with a mean of 1.1 ml. In most cases only one injection of contrast medium has been performed but in 15 cases two injections have been made and in two cases three injections. The contrast medium used was Isopaque 350. This contrast medium has a relatively high iodine content and a relatively low viscosity permitting an adequate rate of injection even through small bore catheters. In 35 cases full size biplane angiocardiography with an Elema Schonander roll film changer operating at 12 pictures per second was used. In 36 patients cine angiography with a 9 inch image intensifier and an Arriflex 35 mm cine camera running at 40 frames per second was used. For the cine angiographic studies the geometrical magnification technique with the magnification factor of approximately 2 was adopted. Exposure factors for the full size biplane angiocardiographies were 800 mA, 0.01 second and 60–75 kV. For the cinerecordings the duration of each cine pulse was 0.003 seconds and the exposure factors were 125 mA and 45–60 kV. High load roentgentubes with rapidly rotating anodes were used. For full size angiocardiography the size of the focal spot was 1.2×1.2 mm and for ciné angiocardiography 0.6×0.6 mm. Linear grids with a 10:1 ratio and high speed, high-contrast film were used for the biplane angiocardiographies. For ciné angiocardiography medium speed, medium grain 35 mm film was used. The definition of the full size recording system varies between 3–4 periods per mm and the definition of the cine recording system is approximately 1.7 periods per mm. When cine angiocardiography was used the injections were monitored by TV fluoroscopy and recorded on video tape.

## RESULTS

*Clinical examination*. Infants with a less severe degree of congenital heart disease were not included in the study. Symptoms of cardiac insufficiency was therefore present in all cases with a respiratory rate more than 50 per minute and hepatomegaly as the most common features. Tachypnoea, being an unspecific sign, was the most common finding. Only two cases had a respiratory rate lower than 50 per minute, one was a valvular pulmonary stenosis and the other a case of vascular ring. An elevated hematocrite value was of some help for the diagnosis if a congenital heart disease was present or if the cardiac failure was secondary to for example a disease of the respiratory tract. Thus a permanently elevated or increasing hematocrite in repeated samples during the first days of life was found to be a good indication of the presence of cyanotic congenital heart disease.

A systolic murmur of organic type was noted and registered with the aid of phonocardiography in 79 cases. A diastolic murmur as well was noted in 14 cases. In one case only a diastolic murmur was noted. In 24 cases no organic heart murmur could be heard or registered.

The second heart sound was found to be pathological with only one component audible and/or visible on the phonocardiogram in 42 cases. It was of interest to note that in 7 out of 9 cases with uncomplicated transposition

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| Total | 103 | 51 | 34 |

and in the septostomy cases the Rashkind catheter number (5 F (8)) was used. In 56 out of the 78 patients where a right heart catheterization was performed the left heart was reached through an atrial septal defect or through foramen ovale.

*Angiocardiography* was performed in 71 patients. Whenever possible the principles of selective angiocardiography have been followed. In a few cases this was impossible and the injections were then made in the right atrium and in one case in the abnormal inferior vena cava. 0.5–2.0 ml of contrast medium per kg of body weight was used for each injection. The total dose of contrast medium varied between 0.5 and 2.0 ml per kg of body weight with a mean of 1.1 ml. In most cases only one injection of contrast medium has been performed but in 15 cases two injections have been made and in two cases three injections. The contrast medium used was Isopaque 350. This contrast medium has a relatively high iodine content and a relatively low viscosity permitting an adequate rate of injection even through small bore catheters. In 35 cases full size biplane angiocardiography with an Elema Schonander roll film changer operating at [illegible] pictures per second was used. In 36 patients cine angiography with a 9 inch image intensifier and an Arriflex 35 mm cine camera running at 40 frames per second was used. For the cine angiographic studies the geometrical magnification technique with the magnification factor of approximately [illegible] was adopted. Exposure factors for the full size biplane angiocardiographies were 400 mA 0.01 second and 60–75 kV. For the cinerecordings the duration of each time pulse was 0.003 seconds and the exposure factors were 125 mA and 45–60 kV. High load roentgentubes with rapidly rotating anodes were used. For full-size angiocardiography the size of the focal spot was 1.2×1.2 mm and for cine angiocardiography 0.6×0.6 mm. Linear grids with a 10:1 ratio and high speed high-contrast film were used for the biplane angiocardiographies. For cine angiocardiography medium speed medium grain 35 mm film was used. The definition of the full size recording system varies between 3–4 periods per mm and the definition of the cine recording system is approximately 1.7 periods per mm. When cine angiocardiography was used the injections were monitored by TV fluoroscopy and recorded on video tape.

## RESULTS

*Clinical examination.* Infants with a less severe degree of congenital heart disease were not included in the study. Symptoms of cardiac insufficiency was therefore present in all cases with a respiratory rate more than 50 per minute and hepatomegaly as the most common features. Tachypnoea being an unspecific sign was the most common finding. Only two cases had a respiratory rate lower than 50 per minute, one was a valvular pulmonary stenosis and the other a case of vascular ring. An elevated hematocrite value was of some help for the diagnosis if a congenital heart disease was present or if the cardiac failure was secondary to for example a disease of the respiratory tract. Thus a permanently elevated or increasing hematocrite in repeated samples during the first days of life was found to be a good indication of the presence of cyanotic congenital heart disease.

A systolic murmur of organic type was noted and registered with the aid of phonocardiography in 79 cases. A diastolic murmur as well was noted in 14 cases. In one case only a diastolic murmur was noted. In 24 cases no organic heart murmur could be heard or registered.

The second heart sound was found to be pathological with only one component audible and/or visible on the phonocardiogram in 42 cases. It was of interest to note that in 7 out of 9 cases with "uncomplicated" transposition

of the great vessels the pulmonary second sound was visible This finding is of help in the differentiation between transposition of the great vessels and pulmonary atresia

If signs of cardiac insufficiency are excluded specific physical findings compatible with congenital heart disease were present in 34 cases out of 41 in the group diagnosed before 2 weeks of life and in 56 cases out of 58 older infants

*Electrocardiography* T wave changes such as flattened T waves and a positive T wave in lead $V_1$ after the age of 3 days were estimated as unspecific but pathological changes Unspecific changes occurred in 7 cases out of 41 investigated before 2 weeks of life and in 7 cases out of 58 in older infants Specific pathological ECG changes were found in 26 out of 41 in the neonatal group and in 51 out of 58 in the infants over 2 weeks of life Thus a normal ECG was found in 8 infants in the small babies but in no case in the older age group

*Conventional roentgen examination* In 41 children examined before 2 weeks of age 25 showed abnormalities on the standard chest roentgen examination indicating cardiac disease whereas the others had no definite abnormalities In 58 examinations in patients over 2 weeks of age 49 had abnormal findings whereas the rest were considered to be within the normal limits This indicates the difficulties in making a diagnosis of cardiac disease on standard chest radiograms in early infancy Even in the infants examined after 2 weeks of age the abnormal findings were generally non-specific such as a general enlargement of the heart, increased vascularity of the lungs and evidence of congestion of the lungs A diagnosis of specific enlargement or hypoplasia of different heart chambers and the large vessels was only occasionally made and even in retrospect diagnosis of the cardiac malformation from the standard roentgenograms was generally impossible in the absence of other information about the patient

*Cardiac catheterization* In 7 cases the diagnosis could be settled with cardiac catheterization only in 2 cases combined with a small manual injection of contrast medium In the other group 71 cases, it is for natural reasons not possible and meaningful to present the contribution of heart catheterization separately from the angiocardiography

*Angiocardiography* A correct and complete diagnosis of the cardiac malformation was made by angiocardiography in 61 cases as verified at operation or at autopsy or both In one of the other a relatively slow injection of contrast medium through a small catheter into the right atrium failed to visualize the abnormal venous return from the lungs A repeated study with injection in the pulmonary artery was planned but not carried out because of the condition of the child In the remaining nine patients the technical quality of the angiocardiograms was adequate and the main cardiac malformation correctly diagnosed but an additional malformation such as a patent ductus a ventricular or atrial septal defect could not be diagnosed Only in two instances the incompleteness of diagnosis was of any consequence for the patient This concerned two patients with pulmonary atresia where the length of the atretic segment was judged to be considerable by angiography whereas in fact there was only a membranous atresia in the valvular region

Only one serious complication to cardiac catheterization and angiocardiography occurred A 10 day old boy at first believed to have a transposition of the great vessels was catheterized with a No 6 5 Rashkind balloon catheter which perforated the myocardial wall of the outflow tract of the right ventricle The child who was found to have a pulmonary membranous atresia was operated a few hours later and hemopericardium and the perforation were found

## DISCUSSION

From this study and also from previous experience it is known that even the differential

diagnosis between a cardiac and extracardiac condition in a new born child may be difficult Even more difficult is the specific diagnosis of a cardiac malformation with the use of clinical examination electrocardiogram and standard chest radiography in the newborn period The explanation for this is partially the complexity of some of the malformations and partially that the typical findings that help to establish the diagnosis by these relatively simple means in older children have not developed in the new born period

Cardiac catheterization and angiocardiography are therefore necessary for the diagnosis of the cardiac malformation in this age group and it seems logical to perform these examinations in every newborn child where a life threatening cardiac malformation is suspected When doing this it is unavoidable that a small number of children with other diseases than cardiac malformations will be catheterized However this seems to be no objection to a more active approach to diagnosis of cardiac disease in the newborn period since the complications in our hands from cardiac catheterization and angiocardiography have been rare The injection of contrast medium and even repeated injections have been tolerated surprisingly well by these severely ill infants which is in contrast to previous investigations indicating that repeated contrast injection should be very hazardous in severely ill children (1) However it must be remembered that these studies were based on use of contrast media with higher toxicity than the media presently used The risk of injection of contrast medium for angiocardiography should not be underestimated however and careful observation of the child after each injection is mandatory

The dangers of cardiac catheterization and angiocardiography in critically ill babies has recently been shown in two North American studies (2 6) In our last 200 cases of infants below the age of one year we have an investigative mortality (within 24 hours after investigation) of one per cent as compared to figures at least five times as high in the materials mentioned above The explanation to this difference is not clear It could be a matter of the condition of the infant when arriving to the hospital or it could be caused by differences in the investigative technique Another explanation could be that the diagnostic procedures at our center is concentrated to as few and experienced hands as possible

As to the angiographic technique in these small children repeated injections in different heart chambers are frequently needed to arrive at a complete diagnosis Considerable experience is necessary for the proper positioning of catheters and of the patient for different injections We have been using both full size biplane angiocardiography and single plane 35 mm cine angiocardiography as recording methods in this material For obvious reasons no comparative studies of the diagnostic value of the two methods have been performed in this material of severely ill infants However in a retrospective evaluation an attempt was made to decide whether one recording method or the other would have provided better results than the method actually used In 65 cases it was considered that both cine angiocardiography or full-size angiocardiography would have provided the essential information for diagnosis and treatment of the patient In four cases full size angiocardiography was considered to be superior and in two cases cine angiocardiography It is clear that both recording systems can provide the clinically important information in most of the cases However in a few cases one method is superior to the other or both methods are necessary to arrive at a complete diagnosis Therefore the angiocardiography room must have the equipment for both recording systems permitting the choice between them according to the findings at catheterization and other known facts about the patient The full-size recording system with its higher definition permits analysis of small anatomical details more accurately than cine angiocardiography However only rarely is this detailed anatomical information needed We therefore prefer to use

cine angiocardiography in combination with video tape recording as the standard method in most cases The use of video tape recording has the additional value of providing possibility for instant viewing and reviewing of the result of an angiocardiography In this way an instant diagnosis can be made in most cases This is particularly valuable in these often severely ill infants where any prolongation of the catheterization procedure or repeating of the procedure is undesirable

In conclusion we recommend an active diagnostic approach to suspected cardiac disease in early infancy All symptomatic patients suspected to have such a condition should be referred for evaluation The diagnosis in these cases is difficult and requires not only special equipment but also a team with experience in this field The study of these patients should be concentrated at a few centers where the experience can be accumulated, thus reducing the hazards of the investigation and increasing the possibilities of getting a correct diagnosis The patients should be considered as emergency cases and cardiac catheterization and angiocardiography should be performed at any time during the 24 hours

## SUMMARY

In a series of 103 consecutive cases of severe cardiac disease in infants the diagnostic value of clinical examination electrocardiogram and conventional roentgen examination was found to be very limited Emergency use of cardiac catheterization and angiocardiography is necessary to achieve a correct diagnosis in these patients

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# BICYCLE ERGOMETER TESTS ON BUILDING APPRENTICES AGED 15–19

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When undertaking medical examination among young people it is important to be able to judge their physical work capacity However there is no recent Swedish normally distributed control group which could be used for judging physical work capacity

Submaximal tests on Swedish boys on bicycle ergometer are recorded only in a few studies see Table 1 (1 6 8 10) In most of these investigations the test group is small and has not been broken down by age Moreover most of these results are rather old and the subjects have been selected

During the spring and autumn of 1967 an investigation was made of how building apprentices at the Stockholm City Trades School adapted to their work (12) In this investigation a medical examination was carried out and the subjects also performed a bicycle ergometer test

## SUBJECTS

Eighty two building apprentices were tested Forty one of them were tested immediately after admittance — new pupils and 41 had completed one of their three years of training— one year pupils The pupils age distribution can be seen in Table 2 Their previous medical history was thoroughly investigated and a medical examination which specially concentrated on the cardiovascular diseases and diseases of the locomotive organs was made In some of the pupils small physical defects and diseases were noticed But none of these was of such a nature that physical work capacity could be affected None of the pupils was advised to discontinue his studies for medical reasons

## METHODS

The work test was performed in the morning at least two hours after breakfast A bicycle ergometer (Monark) with a mechanical braking system and a pedal rate of 50 rpm was used *First the pupils cycled for* six min with a load of 600 kpm/min (100 watts) then the load was increased instantaneously to 900 kpm/min (150 watts) and the cycling continued for six more min The heart rate was measured at the 2nd 4th, 5th and 6th min with a one-channel direct writing ECG recorder An average of the three last readings at each intensity is given unless the difference between the readings is greater than four heart beats If this is the case the heart rate during the sixth min is stated During a few minutes break after the load of 900 kpm/min a prediction was made based upon the heart rates at the submaximal loads of the approximate maximal load the pupil would be able to work at during 5 min (2) Then the subjects continued to cycle with this predicted maximal work load *(generally 1 050 1,200 1 350* or 1,500 kpm/min) until they were exhausted The heart rate was registered once a min and at the end continuously *One* and three min after the cycling stopped two capillary blood samples were taken for analysis of the concentration of blood lactate hereby using the Barker & Summerson method modified by Ström (14)

The oxygen uptake was *predicted from the work* load assuming a constant mechanical efficiency (cf *I Åstrand 1960) For* each individual the heart rates at the two work loads were plotted in a heart rate oxygen uptake diagram A straight line was drawn to the maximal heart rate for an extrapolation of the maximal oxygen uptake see Fig 1

## RESULTS

The height and weight of the examined pupils (see Table 2) has been compared with a normally distributed test group of Swedish boys examined in 1942 (7) The average age/height

Table 1 *Mean heart rates at 600 and 900 kpm/min in earlier Swedish investigations compared to the building apprentices*

E Bengtsson Stockholm 1957 (6) T Karlefors Lund 1966 (10) I Åstrand Forestry apprentices Lycksele and Kosta 1957 (1) and J E Hansson Forestry apprentices 1956–1960 (8)

| | | Number of subjects | Age | Height | Weight | Heart rate 600 kpm/min | Heart rate 900 kpm min |
|---|---|---|---|---|---|---|---|
| | Bengtsson | 7 | 15–20 | | 70 | 130 | 160 |
| | Karlefors | 17 | 16–24 | 177 | 67 | 136 | 160 |
| Åstrand | Lycksele | 27 | 14–21 | | 60 | 123 | 151 |
| | Kosta | 12 | 14–21 | | 60 | 121 | 136 |
| Hansson | 1956–58 | 36 | 16–17 | 173 | 61 | 127 | 153 |
| | 1959–60 | 14 | 16–17 | 174 | 62 | 132 | 156 |
| *Building apprentices* | | *8* | *15* | *169* | *55* | *148* | *178* |
| | | 43 | 16 | 178 | 65 | 136 | 172 |
| | | 21 | 17 | 176 | 61 | 134 | 166 |
| | | 10 | 18–19 | 177 | 65 | 124 | 157 |

values of the building apprentices are above the average values of the 1942 investigation whereas the average height/weight values of the building apprentices largely correspond to the average values of the 1942 group (7) Two building apprentices are above the two sigma limit of the 1942 normally distributed test group as regards age/height whereas six pupils are above and two below the two sigma limit as regards height/weight Pupils aged 18 to 19 years have been grouped together since the number of individuals in these age groups was small and since the physical development between the ages of 18 and 19 is probably rather insignificant

The heart rate at work loads of 600 and 900 kpm/min diminishes with increasing age (Table 3) The difference in heart rate is significant ($p < 0.05$) between 15 and 17 year olds at 600 kpm/min and between 16 and 18–19 year olds at 900 kpm/min Also the maximal heart rate decreases with increasing age although the differences are not significant

Table 2 *Age distribution of new pupils and pupils who had completed one year of training*

| | 15 years | 16 years | 17 years | 18–19 years |
|---|---|---|---|---|
| New pupils | 8 | 28 | 2 | 3 |
| One year pupils | 0 | 15 | 19 | 7 |
| Total | 8 | 43 | 21 | 10 |

In Table 3 the 16 year olds have been divided into two groups One consists of 28 new pupils and the other of 15 one year pupils The heart rate values obtained are lower for the pupils with one year's training and the difference is statistically significant ($p < 0.05$) at 600 and 900 kpm/min

The predicted maximal oxygen uptake (li

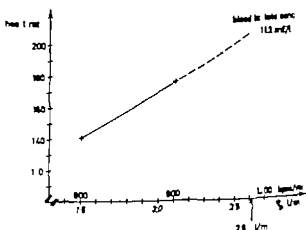



*Fig 1* Example of how the maximal aerobic work capacity has been predicted The subject's heart rate at 600 and 900 kpm/min (corresponding to an oxygen consumption of 1 5 and 2 1 l/min) was 141 and 176/min respectively

The straight line between these points has been extrapolated up to the *maximal heart rate* in this case 207/min The corresponding maximal aerobic capacity was thus 2 6 l/min

*Table 3. Mean standard error of mean and standard deviation of height weight heart rates during work predicted maximal aerobic work capacity and blood lactates in different age groups*

| Age (years) | No of subjects | Height (cm) | Weight (kg) | Heart rate (600 kpm/min) | Heart rate (900 kpm/min) | Maximal heart rate | Predicted max V l/min | Predicted max V ml/kg min | Blood lactate (mEq/l) |
|---|---|---|---|---|---|---|---|---|---|
| 15 | 8 | 169 [illegible]3<br>6 4 | 55 1 9<br>5 5 | 148 7 5<br>0 2 | 178 4 8<br>11 5 | .01 3 9<br>10 9 | 2 5 0 14<br>0 40 | 47 3 3<br>9 2 | 10 1 0 70<br>1 98 |
| 16 | 43 | 178 1 1<br>7 3 | 65 1 6<br>10 5 | 146 2 7<br>17 4 | 17 2 8<br>18 5 | 01 1 4<br>8 9 | 2 7 0 06<br>0 37 | 41 1 1<br>6 9 | 11 1 0 32<br>2 12 |
| 17 | 21 | 176 1 3<br>6 2 | 61 2 0<br>9 2 | 134 2 8<br>13 0 | 166 3 6<br>16 6 | 198 [illegible]3<br>10 6 | 2 8 0 09<br>0 40 | 46 1 3<br>6 0 | 10 6 0 44<br>1 98 |
| 18 19 | 10 | 177 1 9<br>6 0 | 65 1 8<br>5 7 | 124 4 9<br>15 5 | 157 6 5<br>0 5 | 194 1 7<br>5 3 | 2 9 0 24<br>0 75 | 44 2 6<br>8 2 | 11 4 0 54<br>1 72 |

*Table 4. Height and weight heart rates and predicted maximal aerobic work capacity in two groups of 16 year old apprentices one consisting of new pupils and one consisting of one year pupils*

| | No of subjects | Height (cm) | Weight (kg) | Heart rate (600 kpm/min) | Heart rate (900 kpm/min) | Maximal heart rate | Predicted max V l/min | Predicted max V ml/kg min | Blood lactate (mEq/l) |
|---|---|---|---|---|---|---|---|---|---|
| New pupils | .8 | 178 1 3<br>6 8 | 65 2 1<br>11 | 139 2 6<br>13 6 | 176 2 6<br>14 0 | 203 1 6<br>8 6 | 2 6 0 06<br>0 90 | 41 1 2<br>6 5 | 11 0 0 41<br>2 19 |
| One year pupils | 15 | 177 2 1<br>8 3 | 65 2 4<br>9 2 | 179 5 6<br>21 7 | 164 6 1<br>23 5 | 198 2 3<br>8 9 | 2 8 0 12<br>0 47 | 43 2 0<br>7 7 | 11 3 0 53<br>2 07 |

ters/min) shows increasing values with increasing age. However, the differences are not statistically significant.

The blood lactate values obtained after maximal work range from 10.1 to 11.4 mEq/l average and have a range of variance from 7.3 to 16.2 mEq/l.

## DISCUSSION

The persons tested in this investigation are both boys who just left *grundskola* (i.e. secondary modern school or junior high at about 16 years of age) and boys who have completed one year's training at the Builders' School, a training which involves practical work on the building sites. As a result the boys may have acquired some degree of physical training, a possibility which is also indicated by the significantly lower heart rate at submaximal work load of these pupils compared to those of the newly admitted pupils.

In this test group the main growth process measured in height and weight already seems to be completed at age sixteen. Therefore the differences in submaximal heart rate and in predicted maximal aerobic power can be explained by changed physical size only between the 15- and 16-year olds. Possibly the difference obtained from age 16 and onwards could be explained by the fact that the majority of the older boys have completed one year's building apprenticeship and are therefore better trained. Another possible explanation is that although the external dimensions (height and weight) increased very little after age 16, the dimensions of the circulatory system and thereby the work capacity might increase up to the age of 19.

Considering the method used for prediction of the maximal aerobic power, it has some errors. For example it is known that the heart rate may continue to rise somewhat even after the maximal oxygen uptake has been reached (11).

It is known that young people show slightly lower lactate values after maximal work than grown up people (5). Åstrand states 11.6 mEq/l as the average value for 9 boys aged 16 to 18 after maximal work on a treadmill and 6.7 mEq/l as the lower limit for children aged 4 to 20. The values in this investigation are all above the mentioned limit.

1956–60 Hansson tested two groups of forestry worker trainees (14 and 46 subjects) aged 16 to 17 with work loads of 600 and 900 kpm/min, see Table 1 (8). At 600 kpm/min he noted a heart rate of 127 and 132 beats/min respectively, and at 900 kpm/min 153 and 156 beats/min respectively, values which are much lower than those of this test group. This may partly be due to harder physical training in forest work and partly to a real deterioration of physical fitness during the last few years. In other earlier reports on work tests of Swedish boys no separation into age groups has been made and therefore they cannot be compared to these results.

The building apprentices' heart rates at 600 and 900 kpm/min can also be compared to corresponding values for adults. At the health examination of the City of Stockholm in 1962 heart rates of 128 and 149 beats/min at work loads 600 and 900 kpm/min were obtained for a group of men aged 48 to 63 who were a cross section of the population (3). Corresponding values in 1966 for a group of 84 building workers aged 30 to 70 were 122 and 147 beats/min (4). Thus the building apprentices' heart rates are higher than these values also in the 18–19 year olds. This suggests that even the 18–19 year olds have not yet reached a full development of their physical work capacity. Further support for this theory is that earlier studies have shown that the maximal aerobic power increases during the teen ages and reaches its highest value not until the age 20–25 (9, 13).

## SUMMARY

A work test on a bicycle ergometer was carried out on 82 Swedish boys aged 15 to 19. The heart rates were recorded both at the sub-

maximal loads of 600 and 900 kpm/min and at the maximal work load Maximal aerobic power was predicted from submaximal work loads and maximal heart rate The heart rates obtained show decreasing values with increasing age at a given work load This is partly ascribed to physical growth and partly to some training effect noticeable in the older boys who have completed one year of building apprenticeship

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# PATHOLOGICAL FEATURES IN THE DE LANGE SYNDROME

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eatures of this syndrome first recognised by ornelia de Lange (1933) comprise physical id mental retardation with brachycephaly ypertrophy of brows and lashes, small sized inds and feet, proximal position of thumb id thenar eminence low set ears and syndac ly of the toes with the occasional finding of rsute forehead pointed palate curved little nger and humoral micromelia (10) Nowa iys the eponym refers only to the above con tion although the author also described an- her syndrome of mental deficiency associated ith muscular hypertrophy and extrapyrami il motor disturbances (11)

In spite of the increasing interest in the con tion and numerous recent publications, de riptions of pathological changes have been arce and usually incomplete We therefore msider it worth while to present the follow g mainly pathological account of two further ises

*ise 1*

is girl the second child of unrelated 22 year old irents was born after 36 weeks gestation weighing 130 g Her older brother was well at 13 months and ere are no other significant facts in the family story The pregnancy apart from feebleness of foe l movements was normal The placenta was small 54 g) but showed no other abnormality

The patient s appearance was strikingly that of de inge syndrome (fig 1) Her eyebrows met in the idline she had a long thin overhanging upper lip iteverted nostrils with depressed nasal bridge low t ears cleft hard and soft palate webbing on the right side of the neck micrognathia with a promi nent symphysis menti and hypoplastic nipples The palm creases were single Both thumbs were inserted proximally and on the right side the ring and middle fingers were seemingly fused The limbs were hyper tonic and the feet were sharply inverted at the tarso metatarsal joints A loud systolic murmur was aud ible She did not gain weight and died at 4 weeks weighing 1445 g At that time her height was 42 cm and the head circumference 27 5 cm

*Pathological findings*

The *heart* was normal but a left superior vena cava drained into a dilated coronary sinus The *kidneys* weighed only 5 g each and showed nu merous small cysts in the cortex with occa sional foci of dysplasia one of which contained a plaque of bone Some nephrons were present but a number of tubules were dilated and con tained acute inflammatory cells

There was partial bony union of both parie tal and frontal bones along the coronal suture

The *adrenal glands* weighed 2 g each The zona glomerulosa and zona fasciculata were normal although lipoids were slightly reduced in the latter The *thyroid* gland (0 82 g) and one *parathyroid* gland were normal The pi tuitary (0 11 g) showed obvious reduction in the number of basophils although some am photeric cells were of similar size and appear ance but lacked basophil granules Eosinophil and chromophobe cells were present in about normal proportion Occasional cells contained a little P A S positive material The *thymus*



*Fig 1* Case 1 General facial appearance

(0 5 g) was involuted Both *ovaries* were long and thin but were histologically normal

The *brain* was small weighing 188 g (the average normal for age 413 g) the weight of the cerebellum and brainstem accounting for 15 g It was symmetrical and the pattern of convolutions seemed normal The frontal operculae were however underdeveloped the insulae being thereby partially uncovered (Fig 2) The optic *nerves* optic chiasma and less obviously the III and V cranial nerves were small Other cranial nerves had been avulsed during the removal of the brain Coronal sectioning of the brain showed lack of myelin development the entire centrum semiovale remaining somewhat grey in colour and being narrow in relation to the depth of the cortex The brainstem and cerebellum were also reduced in size

Blocks of the frontal temporal parietal and occipital lobes of two levels of the basal ganglia the cerebellum midbrain pons and medulla were embedded in celloidin and sections examined after staining with cresyl violet H V G by the Heidenhain method for myelin and the Holzer method for fibrous glia Other material was embedded in paraffin and stained with haematoxylin and eosin and haematoxylin and Van Gieson Blocks of the cerebrum and brainstem were also cut in frozen sections which were stained with Sudan III P A S acid cresyl violet by the Kulschitsky Pal method for myelin the Bielschowsky silver im

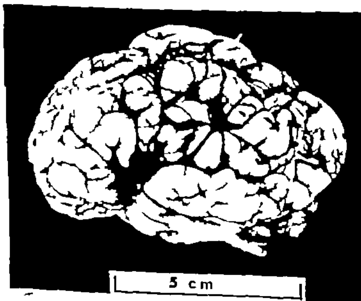



*Fig 2* Case 1 Brain showing exposed insula.

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Features of this syndrome first recognised by Cornelia de Lange (1933) comprise physical and mental retardation with brachycephaly hypertrophy of brows and lashes small sized hands and feet proximal position of thumb and thenar eminence low set ears and syndactyly of the toes with the occasional finding of hirsute forehead pointed palate curved little finger and humoral micromelia (10) Nowadays the eponym refers only to the above condition although the author also described another syndrome of mental deficiency associated with muscular hypertrophy and extrapyramidal motor disturbances (11)

In spite of the increasing interest in the condition and numerous recent publications descriptions of pathological changes have been scarce and usually incomplete We therefore consider it worth while to present the following mainly pathological account of two further cases

*Case 1*

This girl the second child of unrelated 22 year old parents was born after 36 weeks gestation weighing 1530 g Her older brother was well at 13 months and there are no other significant facts in the family history The pregnancy apart from feebleness of foetal movements was normal The placenta was small (354 g) but showed no other abnormality

The patient's appearance was strikingly that of de Lange syndrome (fig 1) Her eyebrows met in the midline she had a long thin overhanging upper lip anteverted nostrils with depressed nasal bridge low set ears cleft hard and soft palate webbing on the right side of the neck micrognathia with a prominent symphysis menti and hypoplastic nipples The palm creases were single Both thumbs were inserted proximally and on the right side the ring and middle fingers were seemingly fused The limbs were hypertonic and the feet were sharply inverted at the tarso metatarsal joints A loud systolic murmur was audible She did not gain weight and died at 4 weeks weighing 1445 g At that time her height was 4[illegible] cm and the head circumference [illegible]7 5 cm

## *Pathological findings*

The *heart* was normal but a left superior vena cava drained into a dilated coronary sinus The *kidneys* weighed only 5 g each and showed numerous small cysts in the cortex with occasional foci of dysplasia one of which contained a plaque of bone Some nephrons were present but a number of tubules were dilated and contained acute inflammatory cells

There was partial bony union of both parietal and frontal bones along the coronal suture

The *adrenal glands* weighed 2 g each The zona glomerulosa and zona fasciculata were normal although lipoids were slightly reduced in the latter The *thyroid* gland (0 82 g) and one *parathyroid* gland were normal The pituitary (0 11 g) showed obvious reduction in the number of basophils although some amphoteric cells were of similar size and appearance but lacked basophil granules Eosinophil and chromophobe cells were present in about normal proportion Occasional cells contained a little P A S positive material The *thymus*



*Fig 1* Case 1 General facial apperance

(0 5 g) was involuted Both *ovaries* were long and thin but were histologically normal

The *brain* was small weighing 188 g (the average normal for age 413 g) the weight of the cerebellum and brainstem accounting for 15 g It was symmetrical and the pattern of convolutions seemed normal The frontal operculae were however underdeveloped the insulae being thereby partially uncovered (Fig 2) The optic *nerves* optic chiasma and less obviously the III and V cranial nerves were small Other cranial nerves had been avulsed during the removal of the brain Coronal sectioning of the brain showed lack of myelin development the entire centrum semiovale remaining somewhat grey in colour and being narrow in relation to the depth of the cortex The brainstem and cerebellum were also reduced in size

Blocks of the frontal temporal parietal and occipital lobes of two levels of the basal ganglia the cerebellum midbrain pons and medulla were embedded in celloidin and sections examined after staining with cresyl violet H V G by the Heidenhain method for myelin and the Holzer method for fibrous glia Other material was embedded in paraffin and stained with haematoxylin and eosin and haematoxylin and Van Gieson Blocks of the cerebrum and brainstem were also cut in frozen sections which were stained with Sudan III P A S acid cresyl violet by the Kulschitsky Pal method for myelin the Bielschowsky silver im

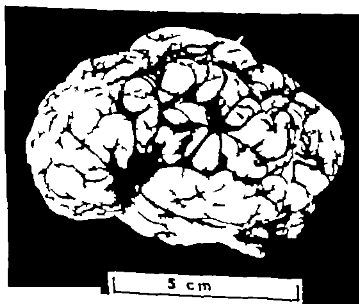



*Fig* Case 1 Brain showing exposed insula

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# PATHOLOGICAL FEATURES IN THE DE LANGE SYNDROME

N E FRANCE L CROME and J M ABRAHAM

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*Pathological findings*

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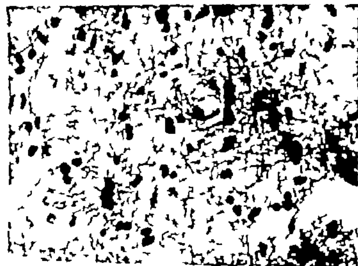


Fig 5 Case 1 Astrocytic over growth and fine fibrous gliosis of white matter Holzer 400



*Fig 6* Case 1 Cerebellar white matter with numerous ectopic neurones. Cresyl violet × 65

cellularly Many axis cylinders could be demonstrated in the white matter by the Bielchowsky method but it proved impossible to decide whether their number was reduced Many ectopic nerve cells were present singly or in small clusters within the white matter The fibrous gliosis was accentuated around the ventricles The basal ganglia presented no ascertainable change but the internal capsule shared in the defective myelination of the centrum semiovale The hypothalamus seemed normal but numerical comparison of neurones with those in normal controls was not attempted

In the relatively small basal and tegmental areas of the *brainstem* the staining of myelin was pale and all the white matter was affected by fibrous gliosis This was particularly dense around the inferior olives around the 4th ventricle and in the median raphe In contrast to the cerebral white matter no sudanophil particles were demonstrable in the gliotic parts of the brainstem The number of nerve cells in some of the cranial nerve nuclei seemed reduced but no attempt was made to test this by counting The *cerebellum* showed fibrous gliosis in the juxtaventricular portion of its white matter Rather diffuse aggregation of primitive neuroblasts was present in the dentate and the roof nuclei (Fig. 6) The white matter in the central part of the cerebellum showed pallor of



pregnation method and the Holzer method for fibrous gliosis

The molecular layer of the *cerebral cortex* showed slight subpial astrocytic overgrowth. The number of neurones in the deeper cortical layers was possibly somewhat reduced and there was in particular a dearth of the larger pyramidal cells. In some areas neurones were arranged in anomalous horizontal rows illustrated in Fig 3. These were formed by a single layer of nerve cells each layer bein separated from the neighbouring ones by relatively acellular spaces. Some of the nerve cells in these horizontal rows were clumped abnormally close to each other

The white matter of the cerebral hemispheres showed a deficiency of myelination and marked diffuse fibrous gliosis (Figs 4 and 5). Numerous sudanophil particles were present in the gliotic areas, both intra and extra

*Fig 3* Case 1 Horizontal stratification in layer 2 of cerebral cortex Cresyl violet × 65





*Fig 4* Case 1 Defective myelination and fibrous gliosis of the white matter *Left* Heidenhain × 1 *Right* Holzer × 1

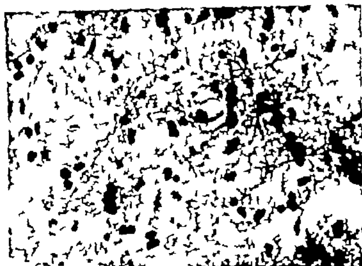


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myelin staining The cerebellar cortex seemed normal although the layer of Bergmann glia was possibly somewhat hyperplastic

*Neurochemistry* (Professor J N Cumings)
Results (other than water) in g/100 g of dry tissue

| | White matter | Cortex |
|---|---|---|
| Total phospholipid | 18 8 | 13 2 |
| Total cholesterol | 7 40 | 4 35 |
| Cholesterol ester | 3 70 | 0 32 |
| Total cerebroside | 2 95 | 1 03 |
| Hexosamine | 0 66 | 0 63 |
| NANA | — | 0 24 |
| Water | 87 8 | 87 3 |

*Thin layer chromatography* Amounts of phospholipids decreased Only one band of sphingomyelin visible otherwise proportions are normal Cholesterol esters present in white matter with only a trace of the esters in the cortex

*Case 2*

This girl was the first child of healthy unrelated 25 year old father and 19 year old mother She was born at term but weighed only 1560 g The mother had been a rubella contact during the first trimester of the pregnancy and attempted unsuccessfully to induce abortion The pregnancy was thereafter uneventful The placenta weighed 283 g and was histologically normal

The appearance of the patient was characteristic (Fig 7) In addition the gums were hyperplastic the palates cleft and the symphysis menti prominent The skull was dolichocephalic with a head circumference of 27 6 cm and widely patent fontanelles Abduction of the hips and extension of the elbows were limited particularly at the right elbow on account of an anterior skin web Two digits (thumb and index) and three (thumb index and middle finger) were present on the right and left hand respectively and the right ulna was absent The nails were well formed The nipples were hypoplastic A blowing systolic murmur was heard over the front and back of the chest The muscles were hypertonic and the patient tended to assume an opisthotonic position The labia minora and clitoris were enlarged

The following investigations performed during the first months of life were normal haemoglobin white cell count platelets serum electrolytes (except potassium and urea which were high) calcium magnesium phosphorus alkaline phosphatase serum proteins (globulin 1 1–2 15 g/100 ml) blood glucose and the electrophoretic strip The Wasserman reaction was negative



*Fig 7* Case 2 Typical facial appearance and deformities of upper limbs

A plasma amino acid screening test showed a slight increase in phenylalanine and tyrosine Phenylalanine levels were recorded at 4 1 and 3 2 mg/100 ml at two and nine months respectively and it was noticed that the levels of other amino acids were also raised Her serum cholesterol was 14? mg/100 ml and protein bound iodine 5 5 μg/100 ml (aged one week) At the age of four months the basal metabolic rate was found to be above average (oxygen consumption 8 6 ml kg in an ambient temperature of 34 C following chloralhydrate sedation) Plasma cortisol was 34 μg/100 ml rising to 52 and 44 μg/100 ml at and 1 hour following the injection of 5 units LVP intramuscularly (aged 2 months)

The electroencephalogram showed diffuse generalised low amplitude fast activity over both hemispheres with no focal or paroxysmal features Chromosome analysis revealed a normal female karyotype

The patient was fed via a naso gastric tube She had intractable diarrhoea and vomited frequently Repeated stool cultures yielded no pathogenic organisms and paper chromatography for sugars in the stools was also negative A barium meal revealed no abnormality of the small or large bowel Sweat sodium concentration was 100 mEq/l She died at the age of nine months weighing 2820 g her height being 18 cm and the head circumference 35 7 cm




*Fig 8* Case 2 Thyroid showing finely vacuolated colloid H and E 200)

*Pathological findings*

There was a universal mesentery but the *gut* had rotated normally The *kidneys* (right 9 g left 11 g) showed a narrow zone immediately beneath the capsule where the interstitial tissue was increased and glomeruli and tubules were reduced in number Micro-cysts in this zone were lined by flat epithelium and sometimes included small glomerular tufts They were frequently surrounded by lamellae of connective tissue

The *adrenal* glands (2 g each) showed a slight reduction of lipoid in the zona fasciculata but were otherwise normal The *thyroid* gland was small Its acini were lined by low columnar or cuboidal epithelium and the colloid stained poorly and showed fine vacuolation (Fig 8) The *pituitary* (0 17 g) had a soft consistency and histologically the pars anterior was very congested and oedematous (Fig 9) Basophil cells were completely absent and there was an apparent increase of eosinophils with relative reduction in the number of chromophobes Occasional cells contained P A S

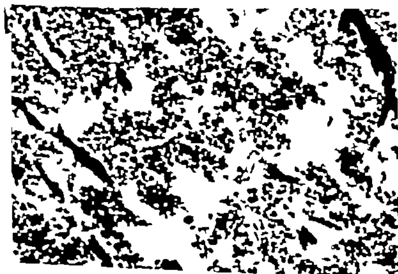


*Fig 9* Case 2 Anterior pituitary showing oedema and preponderence of dark staining eosinophils Heidenhain 200

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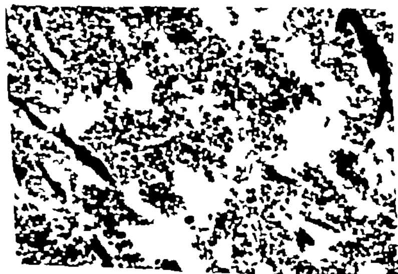


Fig 9 Case 2 Anterior pituitary showing oedema and preponderance of dark staining eosinophils Heidenhain 200

positive material There was a small cyst of the pars intermedia but the pars posterior was normal Both *ovaries* were long and thin but were histologically normal The uterus was hypoplastic and bicornuate

The *brain* weighed 453 g compared with the average for the age of 516 g The brainstem with the cerebellum were relatively large weighing 58 g The brain was markedly elongated measuring 13 cm in length and 9 cm in width The pattern of gyri was normal The optic nerves were small The ventricular system was somewhat dilated The white matter of the cerebral hemispheres and of the brainstem seemed indurated

Histological methods were as described under Case 1

The cerebral cortex presented no unequivocal changes although some neuronal depletion in the superficial layers could not be excluded Pallor of myelin staining and possible retardation of myelination were present in the cerebral white matter A network of faintly staining glial fibrils was discernible in the Holzer preparation Frozen sections stained for fat showed only a small number of lipophages around a few of the blood vessels Some of the vascular spaces contained blood vessels surrounded by rarefied reticular tissue with a few lipophages The basal ganglia were normal The third ventricle was dilated and the hypothalamus compressed Although no detailed study of the hypothalamic nuclei by serial sections with appropriate controls was undertaken there is no *prima facie* evidence of neuronal loss in this area

The brainstem showed considerable reduction in the size of the descending corticopontine and corticospinal tracts The white matter of the brainstem and the cerebellum showed also light fibrous gliosis which was however more evident than in the cerebral white matter Similar gliosis was present in the cerebellar white matter Slight loss of Purkinje cells with overgrowth of Bergman glia was present in the cerebellar cortex The spinal cord showed fine fibrous gliosis of the grey matter Neurochemical examination (Professor J N Cumings) showed some loss of myelin lipids without the presence of esterified cholesterol Appearances were also normal on thin layer chromatography

## DISCUSSION

### *Clinical findings*

The clinical endocrinological and genetic aspects of the syndrome have been reviewed recently (1) The diagnosis depends mainly on the phenotypic expression associated with mental and physical retardation but dermatoglyphics and radiology of the upper limbs are helpful in confirmation Chromosomal studies are usually normal and no consistent biochemical abnormality has been detected The possible correlation between the severity of upper limb deformities and mortality is illustrated by the present cases A similar correlation was suggested by Schuster & Johnson (17) between the epidural ridge pattern and survival the syndrome being less marked in a patient with a relatively normal ridge pattern Hyperammonacidaemia as in Case 2 is not a common manifestation of this syndrome although it has been reported by others (14) this is non specific and, together with the high serum potassium and blood urea in this child may have been secondary to the renal lesion

### *Necropsy findings*

The post mortem findings in 18 patients including the present ones have shown diverse congenital anomalies listed in the table The lesions may involve organs derived from any or all of the three embryonic layers (ectoderm mesoderm and endoderm) Whilst the overall picture is distinctive the spectrum of anomalies is extensive all cases examined pathologically differing somewhat from each other The heart urogenital organs and gastrointestinal tract are affected most frequently Occasionally it was noted that several organs were small but otherwise normal Only one case (7) showed no malformations of internal organs

The cardiovascular anomalies are not uni form There are three examples of ventricular septal defect with or without an atrial septal defect but the remaining seven cases vary from a minor anomaly of the aortic valve (14) to hy poplastic left heart syndrome (6)

Renal hypoplasia dysplasia and cystic change had occurred in eight cases and there are two patients with bilateral hydronephrosis (18 19) Bicornuate or septate uterus has been noted in three cases the ovaries are often long and narrow or hypoplastic and the testes im perfectly descended

Varying degrees of malrotation of the gut usually slight were found in half of the pa tients whilst inclusion of splenic tissue in the tail of pancreas and abnormal lobation of the lungs are usually frequent

*Endocrine glands* Changes in the endocrine system have been observed in eleven patients In two of their cases Schlesinger *et al* (16) considered the basophilic cells of the anterior pituitary to have been replaced completely by amphoteri cells containing scanty P A S po sitive material the histological appearance was similar to that in the present Case 1 Deple tion of basophilic cells was accompanied by almo t complete absence of eosinophilic cells in another patient (5) but by a relative increase of eosinophilic cells in the present Case 2 In one patient (2) the cells of the anterior pitui tary were found in normal proportions but both the anterior and posterior pituitary showed compression hypoplasia by a cyst of Rath ke s pouch The pituitary gland in another case (8) was hypoplastic but histologically normal Hypoplasia of the pituitary gland affecting all elements or diminution in the number of ba ophilic cells suggesting decreased production of thyroid stimulating hormone gonadotroph ins and ACTH are consistent with the finding of evidence of hypopituitarism in some pa tients during life In only two ca es (3 13) were the pituitary glands recorded as normal

*It is known* however that the staining pro pertie of pituitary cells can be equivocal in infancy and childhood and the apparent dis proportion in the number of eosinophil am photeri or basophil cells may well be mislead ing without exhaustive controlled counts of se rial sections of the whole gland

The *thyroid* gland was considered to be nor mal in three cases (9 18 present case 1) but showed some abnormality in the remaining eight cases in whom it was recorded In four of these it was smaller than normal being re duced to as little as one third its normal size (13) Poorly stained finely vacuolated colloid similar to that in Case 1 was noted by Schle singer *et al* (16) whilst scanty or absent col loid was found by others (3 12) The acinar epithelium varied from low cuboidal to high columnar ant interestitial fibro is was present in one child (2) These changes were inter preted as indicative of thyroid hypofunction probably due to underdevelopment

As with the pituitary such reports must be treated with certain caution Apparent struc tural abnormality of the thyroid gland as in the present cases is not uncommon in infants dying from diverse and unrelated causes

In addition to two examples of fusion the *adrenal glands* were considered to be small in five patients and in the present cases their weights were at the lower limit of the normal for age Both the zona fasciculata and zona re ticulosa were considered to be decreased in thickness in one case (2) but this has not been confirmed by other authors These signs of ad reno-cortical insufficiency could be secondary to reduced production of ACTH but it is prob able that they were in some cases the result of terminal infection

Bjorklof *et al* (2) found the *islets of Lang herhans* increased in size and number but pa tients reported by Schlesinger *et al* (16) and the present cases showed no such abnormality The *thymus* was reported to be involuted or hypoplastic in seven cases and a normal *para thyroid* gland was found in Case 1

### *Central nervous system*

Although all recorded cases of de Lange Syn drome have been microcephalic and showed

Table 1 *Congenital anomalies of internal organs*

| | Cardio vascular | Gastro intestinal | Kidneys | Other Anomalies |
|---|---|---|---|---|
| de Lange 1938 (12) | | Non fixation of duo denum and colon | | |
| Richter 1961 (15) | Ventricular septal defect atrial septal defect patent ductus arteriosus | | | |
| Schlesinger *et al* 1963 (16) | | | | |
| Case 1 | Total pulmonary venous drainage into coronary sinus | Non rotation of small and large gut | | Small incompletely descended testes |
| Case 2 | | | | Small incompletely descended testes |
| Ptacek *et al* 1963 (14) | Hypoplasia of one cusp of aortic valve | Non fixation of duodenum and colon | Retarded development | Septate uterus hypoplastic ovaries splenic tissue in pancreas |
| Thornburn 1964 (18) | Small right aortic arch with left patent ductus arteriosus | Partial malrotation | Bilateral hydro nephrosis and hydro-ureter | |
| Gans *et al* 1965 (6) | | | | |
| Case 2 | | Malrotation | | |
| Case 3 | Hypoplastic left heart syndrome atrial septal defect left superior vena cava | | Polycystic | Undescended testes |
| Case 4 | Ventricular septal defect fibro-elastosis of right ventricle | | Multicystic | |
| Hart *et al* 1965 (8) | | Incomplete rotation peritoneal band | | Azygos lobe of right lung trilobate left lung |
| Björklöf *et al* 1965 (2) | Small heart | | Small | Bicornuate uterus long narrow ovaries spleen fused with pancreas |
| Choo *et al* 1965 (3) | Small heart Right common carotid and subclavian arteries have separate origins | Non fixation of colon | Small | Hypoplastic ovaries, accessory lobe of spleen extending into pancreas |
| Falek *et al* 1966 (5) | Ventricular septal defect atrial septal defect over riding aorta | | | |
| Zappella 1966 (19) | | | Bilateral hydro nephrosis | Accessory spleen atrophic undescended testes |
| McArthur *et al* 1967 (13) | | Universal mesentery Meckel's diverticulum | | Undescended testes abnormal lobation of lungs |
| Huang *et al* 1967 (9) | Patent ductus arteriosus | Universal mesentery | Hypoplasia dysplasia cystic | Hypoplasia of lungs trilobate left lung |
| This report | | | | |
| Case 1 | Left superior vena cava | Universal mesentery | Hypoplasia dysplasia cystic | Long narrow ovaries |
| Case 2 | | | Hypoplasia dysplasia cystic | Hypoplastic bicornuate uterus long narrow ovaries |

signs of mental retardation a full neuropathological examination appears to have been undertaken in only one case (12) Some information on the state of the brain in 9 other cases is however al o available (3 7 8 14 15 16 19) Apart from terminal and incidental changes such as subarachnoid haemorrhage and sinus thrombosis the main reported anomalies were microcephaly simplified pattern f convolutions and anomalous myelination interpreted as either retardation or degeneration In one case (7) there was also a deficiency f nerve cells in the anterior horn of the cervical egment of the spinal cord One case is reported to have had hydrocephalus Hart et al 8) men ion very briefly a marked generali ed reduction in the number of the cerebral ganglion cells but this is not convincingly demonstrat d in the accompanying photograph When allowan e is made for differences in the method of study its scope and the extent of reported detail a certain morphological similarity is detectable in all cases and the changes can perhaps be characterised as microcephaly with evidence of underdevelopment particularly of the white matter

As in some of the previous cases the main anomalies of Case 1 above viz, microcephaly underdevelopment of the frontal operculae ectopic nerve cells in the cerebral and cerebellar white matter immaturity of myelin formation can all be regarded a normal stages of development at earlier stages of gestation The anomalous horizontal stratification of some areas of the cerebral cortex in Case 1 has been previously observed and discussed in a spastic microcephalic child aged 2 1/4 years by Dodgson (4) who saw similar stratification in an immature foetus of 26 weeks gestation Thus this change is also consistent with an arrest or retardation in the structural maturation of the brain The widespread fibrous gliosis of the white matter abundance of sudanophil debris and the neurochemical findings in Case 1 suggest however associated breakdown of myelin as in sudanophil leucodystrophy

The pathological changes in Case 2 were not as obvious as those of Case 1 Microcephaly was milder and fibrous gliosis less evident Retardation of myelination was present but there was no evidence of myelin breakdown The appearances were those of burned out encephalopathy Thus if the same pathogenetic processes have operated in the 2 cases one may assume that degeneration of myelin is time limited and possibly non progressive occurring only in early infancy and perhaps intrauterine life Reference to published reports is not conclusive in regard to this problem Demyelination has been mentioned by some of the previous workers (12 14 16) but the precise nature of the changes cannot be established on account of the already mentioned lack of detail and differences in the methods of study

It has been suggested (1) that some endocrine abnormalities observed in the de Lange syndrome are compatible with dysfunction of the hypothalamic area No lesions were observed in the hypothalami of the pre ent cases by the methods employed but hypothalamic dysfunction as part of the more widespread cerebral involvement in the 2 cases cannot be ruled out by morphological study

## SUMMARY

Two girls with Cornelia de Lange Syndrome dying at the age of 4 weeks and 9 months respectively are described

Publ shed examples where necropsy was performed are reviewed and the congenital anomalies of internal organs are summarised

Although some neuropathological features probably result from arrest or retardation of maturation there was evidence in the younger infant of associated breakdown of myelin

Morphological changes of pituitary thyroid and adrenal glands are frequent but no link with hypothalamic lesions has been found

## ACKNOWLEDGEMENTS

We are grateful to Dr B Levin for the biochemical investigations

Table 1 *Congenital anomalies of internal organs*

| | Cardio vascular | Gastro-intestinal | Kidneys | Other Anomalies |
|---|---|---|---|---|
| de Lange 1938 (12) | | Non fixation of duodenum and colon | | |
| Richter 1961 (15) | Ventricular septal defect atrial septal defect patent ductus arteriosus | | | |
| Schlesinger *et al* 1963 (16) | | | | |
| Case 1 | Total pulmonary venous drainage into coronary sinus | Non rotation of small and large gut | | Small incompletely descended testes |
| Case 2 | | | | Small incompletely descended testes |
| Ptacek *et al* 1963 (14) | Hypoplasia of one cusp of aortic valve | Non fixation of duodenum and colon | Retarded development | Septate uterus hypoplastic ovaries splenic tissue in pancreas |
| Thornburn 1964 (18) | Small right aortic arch with left patent ductus arteriosus | Partial malrotation | Bilateral hydronephrosis and hydro ureter | |
| Gans *et al* 1965 (6) | | | | |
| Case 2 | | Malrotation | | |
| Case 3 | Hypoplastic left heart syndrome atrial septal defect left superior vena cava | | Polycystic | Undescended testes |
| Case 4 | Ventricular septal defect fibro-elastosis of right ventricle | | Multicystic | |
| *Hart et al 1965 (8)* | | *Incomplete rotation* peritoneal band | | Azygos lobe of right lung trilobate left lung |
| Björklof *et al* 1965 (2) | Small heart | | Small | Bicornuate uterus long narrow ovaries spleen fused with pancreas |
| *Choo et al 1965 (3)* | *Small heart Right* common carotid and subclavian arteries have separate origins | *Non fixation of colon* | *Small* | Hypoplastic ovaries, accessory lobe of spleen extending into pancreas |
| Falek *et al* 1966 (5) | Ventricular septal defect atrial septal defect over riding aorta | | | |
| Zappella 1966 (19) | | | Bilateral hydronephrosis | Accessory spleen atrophic undescended testes |
| McArthur *et al* 1967 (13) | | Universal mesentery Meckel's diverticulum | | Undescended testes abnormal lobation of lungs |
| Huang *et al* 1967 (9) | Patent ductus arteriosus | Universal mesentery | Hypoplasia dysplasia cystic | Hypoplasia of lungs trilobate left lung |
| This report | | | | |
| Case 1 | Left superior vena cava | Universal mesentery | Hypoplasia dysplasia cystic | Long narrow ovaries |
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# INTRAVENOUS GLUCOSE TOLERANCE PLASMA INSULIN FREE FATTY ACIDS AND β-HYDROXYBUTYRATE IN UNDERWEIGHT NEWBORN INFANTS

JOHAN C H GENTZ RICHARD WARRNER BENGT E H PERSSON[1] AND MARVIN CORNBLATH[2]

*From the Department of Pediatrics University of Illinois College of Medicine Chicago Ill USA*

olerance to intravenous and oral glucose has een studied in normal full term newborns and the offspring of diabetic or gestational dia etic mothers (1 4 5 8 9 14 18 29 33) he removal rate of intravenously adminis ered glucose was slower in the normal new orn infant than that in older children and dults (8) The insulin response was variable ut the normal infant appeared capable of re easing insulin in response to hyperglycemia (14 29 33) Infants of insulin treated diabetics and noninsulin treated gestational diabetics cleared intravenously administered glucose more rapidly (9) and the latter showed higher plasma insulin levels more promptly than did normal newborn babies (1 14)

Recently serum insulin levels have been in vestigated in premature babies during their first 24 hours of life (12) In this study glucose infusions (2 5 g/kg/2 hrs) produced glucose levels over 250 mg/100 ml but resulted only in slight elevations of the serum insulin How ever after an infusion of a mixture of essential amino acids (2 5 g/30 min) a striking increase in serum insulin was seen while the blood sugar either remained unchanged or rose slowly

The newborn who is small for gestational age not only has a significant incidence of hy poglycemia (6 7) but also of transient diabetes mellitus (10) The reasons for this instability in carbohydrate metabolism have not been fully understood In infants with symptomatic hypoglycemia the removal rate of intrave nously administered glucose has been found to be very fast (mean $k_t = 3\ 03$ /min) (11) The plasma insulin values measured at the time of the hypoglycemia have been compa rable to or slightly elevated over fasting levels found in normal babies (6) Thus the infants with symptomatic hypoglycemia had relatively high insulin concentrations at the time their blood glucose values were low The insulin val ues in one newborn infant with transient dia betes were also similar to normal fasting levels and thus were inappropriate for the degree of hyperglycemia present (10)

The plasma concentration of free fatty acids (FFA) during the first days of life in under weight babies have been reported to be higher than those in normal newborns (23) At the same age the plasma level of ketone bodies measured as acetone were increased (20)

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This work was supported in part by Research Grants (HD-03959-01 and HD 03953-01) a Training Grant (TS-04) from the National Institutes of Child Health and Human Development, U.S.P.H.S and from Semper Fond för Näringsforskning

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Tolerance to intravenous and oral glucose has been studied in normal full term newborns and in the offspring of diabetic or gestational diabetic mothers (1 4 5 8 9 14 18 29 33) The removal rate of intravenously administered glucose was slower in the normal newborn infant than that in older children and adults (8) The insulin response was variable but the normal infant appeared capable of releasing insulin in response to hyperglycemia (14 29 33) Infants of insulin treated diabetics and noninsulin treated gestational diabetics cleared intravenously administered glucose more rapidly (9) and the latter showed higher plasma insulin levels more promptly than did normal newborn babies (1 14)

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Table 3 *SGA and AGA infants grouped according to insulin response after the glucose load*

| Groups | Number of infants | Gestational age weeks | | Birth weight g | | Age at test hours | | Fasting blood sugar mg/100 ml | | Fasting plasma insulin μU/ml | | Glucose tolerance expressed as $k_t$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mean | range | mean | range | mean | range | mean | range | mean | range | mean | ±1 S D | range |
| *SGA* | | | | | | | | | | | | | | |
| Double peak | 10 | 38 | 34–40 | 1840 | 1360–2300 | 37 | 12–93 | 53 | 16–93 | 8 | <4–15 | 0 97 | 0 56 | 0 29–1 98 |
| One peak | 4 | 38½ | 38–39 | 1880 | 1650–2000 | 41 | 6–70 | 42 | 31–66 | 11 | 5–17 | 1 29 | 0 15 | 1 51–1 30 |
| No response | 6 | 37 | 36–39 | 1880 | 1530–2030 | 42 | 15–82 | 43 | 7–70 | 4 | <4–6 | 1 07 | 0 29 | 0 55–1 30 |
| *AGA* | | | | | | | | | | | | | | |
| Double peak | 10 | 32 | 28–35 | 1820 | 1450–2000 | 31 | 5–66 | 37 | 21–55 | 11 | <4–18 | 1 10 | 0 18 | 0 77–1 40 |
| One peak | 3 | 33 | 32–36 | 1940 | 1930–1980 | 37 | 14–76 | 50 | 46–59 | 13 | 4–23 | 1 56 | 0 61 | 1 17–2 27 |
| No response | 2 | 31½ | 31–32 | 1860 | 1710–2020 | 38 | 29–47 | 43 | 36–50 | 4 | <4 <4 | 1 32 | 0 17 | 1 2 –1 44 |

250 mg/100 ml in all groups The glucose values had not returned to the fasting level after 60 minutes in any of the infants

The removal rates of glucose from blood were expressed as mean $k_t$ (±S D and range) (Table 2) In SGA infants less than 24 hours of age the mean $k_t$ was significantly lower ($p<0\ 05$) than that in AGA infants In all groups 24 to 48 hours and >48 hours of age the mean $k_t$ values were similar In the SGA group a significant difference in mean $k_t$-values was found between those <24 and those 24 to 48 hours old ($p<0\ 05$) and between those <24 and >48 hours of age ($p<0\ 01$) In the AGA infants there were no significant differences between the mean $k_t$ values of any age groups (Table 2)

*Plasma insulin values*

The mean (±S D and range) fasting plasma insulin values are given in Table 2 and Fig 1 The mean fasting values in both the SGA and AGA infants were similar with wide ranges within the different age groups The AGA babies tended to have somewhat higher mean fasting levels than the corresponding SGA age group but the differences were not significant There was no correlation between individual fasting glucose and insulin values

Following glucose the mean plasma insulin responses of AGA babies in all age groups were greater than that of corresponding SGA babies The differences were most marked in the infants of less than 24 hours of age but were not statistically significant (Fig 1)

In most infants 14 SGA and 13 AGA the administration of the glucose produced an immediate increase in the plasma insulin values at 2 and 5 minutes In 10 SGA and in 10 AGA babies the insulin values then decreased at 10 minutes below 2 and 5 minutes values These 20 showed a second peak at 60 minutes which in 12 exceeded the first peak value seen at 2 minutes In 4 SGA and in 3 AGA infants, only one peak value occurred at 2 minutes and was higher than that seen when two peaks

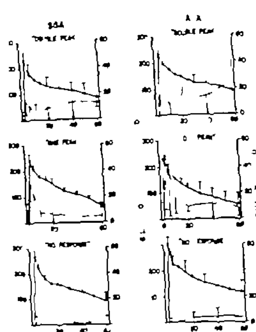


*Fig. 2.* The mean (±1 S.D.) increments for blood glucose (●——●) and plasma insulin (○ ○) are given for the SGA and AGA infants grouped according to the pattern of their insulin response to an acute glucose load (1 g/kg of body weight/2 min).

sisting of 6 SGA and 2 AGA did not show any appreciable increase in plasma insulin after glucose. The type of insulin response could not be correlated with gestational age, birthweight or age at test in either group of babies. There was no relationship between the removal rate of glucose ($k_t$) and the type or height of the insulin response.

*Infants grouped according to their insulin response*

The SGA and AGA infants were then grouped according to their different insulin responses following the glucose load (Table 3). The groups could be subdivided into those showing a double peak, one peak and no response in their peripheral plasma insulin values. The mean birthweight, age at time of test and fasting blood glucose were similar for all groups (Table 3). Within AGA and SGA groups the gestational ages were similar re-

Table 4. *Mean values (±1 S.D.) for plasma FFA and β hydroxybutyrate before and after the glucose load in the SGA and AGA infants grouped according to age*

For statistical comparison between the different mean values see text

| Age group (hours) | Number of infants | Parameter mmol/l | Time in minutes 0 | 2 | 5 | 10 | 20 | 40 | 60 |
|---|---|---|---|---|---|---|---|---|---|
| *SGA* | | | | | | | | | |
| <24 | 6 | FFA | 1.60 ± 0.74 | 1.44 ± 0.50 | 1.38 ± 0.62 | 1.49 ± 0.23 | 1.5 ± 0.27 | 1.3? ± 0.49 | 1.28 ± 0.51 |
| 24–48 | 5 | FFA | 1.91 ± 0.79 | 1.98 ± 0.65 | 2.03 ± 0.6? | 2.45 ± 0.14 | 1.72 ± 0.80 | 1.44 ± 0.79 | 1. 4 ± 0.57 |
| >48 | 6 | FFA | 1. 0 ± 0.50 | 1.57 ± 0.64 | 2.?3 ± 0.35 | 1.73 ± 0.79 | 1.34 ± 0.60 | 1.10 ± 0.5? | 0.94 ± 0.3? |
| <24 | 4 | β OH butyrate | 0.454 ± 0.?93 | 0.4 4 ± 0.346 | 0.456 ± 0.394 | 0.465 ± 0.409 | 0.490 ± 0.470 | 0.576 ± 0.483 | 0.339 ± 0.344 |
| 24–48 | 4 | β OH butyrate | 0.380 ± 0.333 | 0.383 ± 0.336 | 0.380 + 0.334 | 0.371 ± 0.3 5 | 0.344 ± 0.334 | 0.312 ± 0.356 | 0.?93 ± 0.368 |
| >48 | 6 | β OH butyrate | 0.673 ± 0.364 | 0.964 _ 0.669 | 0.592 + 0.371 | 0.760 ± 0.585 | 0.755 ± 0.531 | 0.503 ± 0. 44 | 0.437 ± 0.274 |
| *AGA* | | | | | | | | | |
| <24 | 7 | FFA | 0.87 ± 0.28 | 0.96 ± 0.31 | 0.84 ± 0.23 | 0.81 ± 0.31 | 0.80 ± 0.26 | 0.64 ± 0.16 | 0.58 ± 0. 0 |
| 24–48 | 4 | FFA | 1. 4 ± 0.39 | 1. ? ± 0.16 | 1.39 ± 0.18 | 1.40 ± 0.14 | 1.?6 ± 0.78 | 0.95 ± 0.14 | 0.78 ± 0. 7 |
| >48 | | FFA | 2.79 and 1.04 | ?.15 and 1.69 | — and 1.88 | — — | 2.17 and 1.39 | 1.83 and 1.05 | 1.80 and 0.93 |
| <24 | 6 | β OH butyrate | 0. 08 ± 0.170 | 0. 07 ± 0.167 | 0. 18 ± 0.170 | 0.?12 ± 0.170 | 0.165 ± 0.1 6 | 0.141 ± 0.063 | 0.093 + 0.063 |
| 24–48 | 4 | β OH butyrate | 0.346 ± 0. 0 | 0.369 ± 0. 07 | 0.377 ± 0. 04 | 0.394 ± 0.?0? | 0.3?0 ± 0.1?? | 0.303 ± 0.181 | 0. 50 ± 0.134 |
| >48 | | β OH butyrate | 0.081 and 0.500 | 0.063 and 0.531 | 0.066 and 0.577 | 0.071 and 0.456 | 0.079 and 0.473 | 0.078 and 0.390 | 0.073 and 0.?00 |

Table 3 *SGA and AGA infants grouped according to insulin response after the glucose load*

| Groups | Number of infants | Gestational age weeks mean | Gestational age weeks range | Birth weight g mean | Birth weight g range | Age at test hours mean | Age at test hours range | Fasting blood sugar mg/100 ml mean | Fasting blood sugar mg/100 ml range | Fasting plasma insulin µU/ml mean | Fasting plasma insulin µU/ml range | Glucose tolerance expressed as $k_t$ mean | Glucose tolerance expressed as $k_t$ ±1 S.D. | Glucose tolerance expressed as $k_t$ range |
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*Plasma insulin values*

The mean (±S D and range) fasting plasma insulin values are given in Table 2 and Fig 1 The mean fasting values in both the SGA and AGA infants were similar with wide ranges within the different age groups The AGA babies tended to have somewhat higher mean fasting levels than the corresponding SGA age group but the differences were not significant There was no correlation between individual fasting glucose and insulin values

Following glucose the mean plasma insulin responses of AGA babies in all age groups were greater than that of corresponding SGA babies The differences were most marked in the infants of less than 24 hours of age but were not statistically significant (Fig 1)

In most infants 14 SGA and 13 AGA the administration of the glucose produced an immediate increase in the plasma insulin values at 2 and 5 minutes In 10 SGA and in 10 AGA babies the insulin values then decreased at 10 minutes below 2 and 5 minutes values These 20 showed a second peak at 60 minutes which in 12 exceeded the first peak value seen at 2 minutes In 4 SGA and in 3 AGA infants only one peak value occurred at 2 minutes and was higher than that seen when two peaks were present A third group of infants con

## DISCUSSION

The fasting levels of glucose tend to be lower in low birthweight infants than in full sized babies (7). In the present study the mean glucose values after a 3 hour fast were similar to those reported earlier (5, 8) and did not differ between the groups of SGA and AGA infants.

In both the SGA and the AGA babies the disappearance rates of intravenously administered glucose were slower during the first 24 hours of life than those during the following days. This change in glucose tolerance was significant in the SGA but not in the AGA infants. The $k_t$-values were similar to those reported in normal full term (4, 8, 14) and in premature infants (5) using the same technique (8). There was no correlation between $k_t$-values and the fasting blood glucose levels.

The plasma insulin levels reported in normal newborn infants vary in different laboratories (14, 15, 21, 24, 29, 33, 34). Isle et al (14) have recently reported plasma insulin values in newborn infants of normal and gestational diabetic mothers using the immunoassay of Hales & Randle. In normal infants at 2 hours of age the mean plasma insulin value was 49 μU/ml. In contrast Milner & Hales using the same method reported a mean value of 9 μU/ml in blood from the umbilical vein at birth in 31 normal babies (21). The ranges however were wide in both studies. The insulin values reported by Milner & Hales were similar to those found in this laboratory in normal full term infants (29) using the method of Morgan & Lazarow (22).

The individual infants in both the SGA and the AGA group demonstrated three distinct patterns of insulin response as measured in peripheral plasma. In 20 of 35 infants the pattern was that of a double peaked response with the maximum value at 60 minutes after glucose in 1. The first peak could easily be missed if samples were not obtained within 5 minutes after the injection of glucose. This pattern of insulin response has been described by Isle et al (14) in normal newborn infants. A similar observation has recently been made in full term foetuses in response to maternal administration of glucose. Foetal capillary samples were obtained by Saling's method before onset of labour (3).

It has been suggested that the secondary peak may be the result of a prolonged continuous release of insulin in response to a continuing stimulus (14). However the blood glucose values at 40 and 60 minutes after the administration of glucose were not significantly different in this group of infants from those in the groups exhibiting the one peak or no response insulin curves. Recently Kanazawa et al (16) have measured plasma insulin values after glucose administration in simultaneously obtained blood samples from femoral hepatic and pancreatic veins in dogs. After injection of glucose (1 g/kg/10 min) into the femoral vein the insulin concentration in plasma from the pancreatic vein showed two or more peaks in all experiments. The initial peak was observed just after glucose injection was stopped and the secondary peaks reaching heights exceeding the initial peak appeared between 20 and 90 minutes later. Frequently these secondary peaks were not apparent in the insulin values from the samples of blood from the peripheral and the hepatic veins. Dogs which exhibited a double peak insulin curve had a slower removal rate of glucose than those with a single peak response. The better though not significantly different glucose tolerance among both the SGA and AGA infants who showed a smooth single peak insulin curve would be compatible with these finding. The clearance rate of glucose in offspring of non insulin treated gestational diabetic mothers was significantly faster than in normal newborns and was also associated with a peak insulin at 2 minutes (14). This type of insulin response was also seen in normal adolescents and adults (27, 30). In adolescents there seems to be a close relation between blood glucose and corresponding insulin values after the intravenous glucose load (27). The rate of glucose disappearance was related to the magnitude of insulin response and increasing $k_t$-values cor

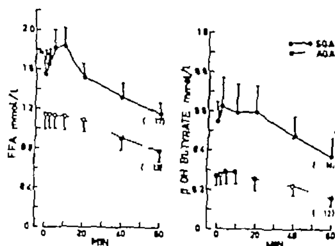



*Fig 3* The FFA and β hydroxybutyrate response to a glucose load (1 g/kg of body weight/2 min) in the SGA (●——●) and in the AGA (○ ○) infants (Mean values ±1 S D) *N* – refers to the number of infants in each group Degree of statistical difference between the mean FFA and β hydroxybutyrate values of the SGA and AGA infants $p<0.01$ $p<0.05$ • not significant – N S

gardless of insulin response The mean fasting values of plasma insulin were lower in the 8 infants in the no response group than those in any other group A significant difference in fasting insulin values ($p<0.025$) was found between the no response and one peak group of the SGA infants (Table 3)

The mean increments (1 S D) for blood glucose and plasma insulin after the glucose load are shown in Fig 2 The blood glucose values did not differ significantly between the groups at any given time

The mean insulin levels in the double peak group were higher at 60 minutes than those after 2 minutes but the difference was not significant The peak values at 2 minutes in the one peak groups were the highest values seen in any of the groups

The mean values for $k_t$ (±1 S D and range) for the different groups are shown in Table 3 The mean $k_t$ values were not significantly different between any groups The $k_t$ values did not correlate with the magnitude of the insulin response There was no correlation between individual values for glucose and insulin during the glucose load

*Plasma FFA and β hydroxybutyrate*

The mean values (±1 S D) for plasma FFA and β hydroxybutyrate before and after the glucose load are given in Table 4 The fasting FFA and β hydroxybutyrate values and those at 60 minutes were not significantly different in either the SGA or the AGA groups (Table 4) The decrease of FFA and β hydroxybutyrate from fasting to 60 minutes was not significant in any of the SGA or AGA groups

In only the age group <24 hours the mean fasting FFA and those at 60 minutes were significantly higher ($p<0.05$) in the SGA than those in the AGA infants The β hydroxybutyrate values were not significantly different between the SGA and AGA babies at any time in any age group

In Fig 3 the fasting values of FFA and β hydroxybutyrate and the changes following the administration of glucose are given for the entire groups of SGA and AGA infants regardless of age The mean FFA values were significantly different between the SGA and AGA infants at 2 5 10 20 40 and 60 minutes but not at zero time (Fig 3) The mean fasting values as well as those at 2 10 20, and 40 minutes for β-hydroxybutyrate in the SGA group were significantly higher than those in the AGA babies The values were not significantly different at 60 minutes

The decreases in FFA and β hydroxybutyrate following the glucose load were not significant for either group of infants

There was no correlation between the individual fasting FFA and $k_t$ values nor between the individual fasting FFA and the fasting insulin levels in either the SGA or the AGA infants The decrease in FFA and β hydroxybutyrate from fasting to 60 minutes after the glucose load was not significantly greater in those babies with a single peak than in those with a no response insulin curve

## DISCUSSION

The fasting levels of glucose tend to be lower in low birthweight infants than in full sized babies (7). In the present study the mean glucose values after a 3 hour fast were similar to those reported earlier (5, 8) and did not differ between the groups of SGA and AGA infants.

In both the SGA and the AGA babies the disappearance rates of intravenously administered glucose were slower during the first 24 hours of life than those during the following days. This change in glucose tolerance was significant in the SGA but not in the AGA infants. The $k_r$-values were similar to those reported in normal full term (4, 8, 14) and in premature infants (5) using the same technique (8). There was no correlation between $k_r$-values and the fasting blood glucose levels.

The plasma insulin levels reported in normal newborn infants vary in different laboratories (14, 15, 21, 24, 29, 33, 34). Isles *et al.* (14) have recently reported plasma insulin values in newborn infants of normal and gestational diabetic mothers using the immunoassay of Hales & Randle. In normal infants at 2 hours of age the mean plasma insulin value was 49 μU/ml. In contrast, Milner & Hales using the same method reported a mean value of 9 μU/ml in blood from the umbilical vein at birth in 31 normal babies (21). The ranges however were wide in both studies. The insulin values reported by Milner & Hales were similar to those found in this laboratory in normal full term infants (29) using the method of Morgan & Lazarow (22).

The individual infants in both the SGA and the AGA group demonstrated three distinct patterns of insulin response as measured in peripheral plasma. In 20 of 35 infants the pattern was that of a double peaked response with the maximum value at 60 minutes after glucose in 1?. The first peak could easily be missed if samples were not obtained within 5 minutes after the injection of glucose. This pattern of insulin response has been described by Isles *et al.* (14) in normal newborn infants. A similar observation has recently been made in full term foetuses in response to maternal administration of glucose. Foetal capillary samples were obtained by Saling's method before onset of labour (3).

It has been suggested that the secondary peak may be the result of a prolonged continuous release of insulin in response to a continuing stimulus (14). However the blood glucose values at 40 and 60 minutes after the administration of glucose were not significantly different in this group of infants from those in the group exhibiting the "one peak" or "no response" insulin curves. Recently Kanazawa *et al.* (16) have measured plasma insulin values after glucose administration in simultaneously obtained blood samples from femoral, hepatic and pancreatic veins in dogs. After injection of glucose (1 g/kg/10 min) into the femoral vein the insulin concentration in plasma from the pancreatic vein showed two or more peaks in all experiments. The initial peak was observed just after glucose injection was stopped and the secondary peaks, reaching heights exceeding the initial peak, appeared between 20 and 90 minutes later. Frequently these secondary peaks were not apparent in the insulin values from the samples of blood from the peripheral and the hepatic veins. Dogs which exhibited a double peak insulin curve had a slower removal rate of glucose than those with a single peak response. The better though not significantly different glucose tolerance among both the SGA and AGA infants who showed a smooth single peak insulin curve would be compatible with these findings. The clearance rate of glucose in offspring of non insulin treated gestational diabetic mothers was significantly faster than in normal newborns and was also associated with a peak insulin at 2 minutes (14). This type of insulin response was also seen in normal adolescents and adults (27, 30). In adolescents there seems to be a close relation between blood glucose and corresponding insulin values after the intravenous glucose load (27). The rate of glucose disappearance was related to the magnitude of insulin response and increasing $k_r$-values cor

responded to higher peak values for plasma insulin (30)

The infants with the no response insulin curves had significantly lower fasting insulin levels than any other group although the blood glucose values were not significantly different The pattern of the insulin response in these infants was the same as seen in adult diabetic subjects (25) A poor glucose tolerance would therefore be expected but the mean $k_t$ values were not significantly different from those seen in the group with a double peak pattern Insulin as measured in peripheral plasma was obviously not the only factor effecting the removal of glucose from the circulation of the newborn infant This is further supported by the findings that the human full term foetus cleared glucose after intravenous glucose load to the mother at the same rate whether the foetus showed an insulin response or not (3) However insulin levels in peripheral plasma may not reflect only the secretion of insulin but may also be dependent on other factors such as liver degradation blood flow and local inactivation Therefore the different patterns in insulin response may result from a balance of many factors, one only being the quantity of insulin secreted

In the SGA groups two infants at 17 and 33 hours of age showed hypoglucosemia at the time of testing Their blood glucose values were 16 and 7 mg/100 ml and their plasma insulins 9 and 5 $\mu$U/ml respectively Both babies were free of symptoms that could be attributed to hypoglycemia The clearance rate of glucose was normal in both infants ($k_t$ = 0 7 and 1 26 /min) with insulin curves of the double peak pattern in one and no response in the other These findings were in contrast with the very fast clearance rate of glucose (mean $k_t$ 3 03/min) in infants with symptomatic hypoglycemia but in accordance with a normal disappearance rate in infants with asymptomatic hypoglycemia (11)

Both the SGA and AGA infants have their peak values for FFA in the age group 24 to 48 hours (Table 4) This pattern for FFA concentration after birth is comparable to that reported in normal full term babies (26) The mean values for FFA in all age group of both the AGA and SGA infants were higher than those reported in full term infants (26) When grouped together the SGA babies have higher mean values for FFA than those in the AGA infants (Fig 3) These findings are similar to the results previously reported in dysmature infants (23)

The $\beta$ hydroxybutyrate concentration reached a peak value in the age group >48 hours (Table 4) in the SGA The later peak in $\beta$ hydroxybutyrate concentration as compared to that of FFA has been reported in full term infants (28) In the SGA infants the mean fasting $\beta$ hydroxybutyrate values were significantly higher than those in the AGA infants (Fig 3) but the levels for both groups were within the range observed in normal full term babies (28 36) Thus there was an unexpected discrepancy between the very high FFA levels and the rather low concentration of $\beta$ hydroxybutyrate in all underweight babies

The drop of the FFA and $\beta$ hydroxybutyrate concentrations after the glucose load was small but similar in the SGA and AGA infants (Fig 3) The change in $\beta$ hydroxybutyrate concentration paralleled that of the FFA This moderate drop in the FFA level after a glucose load was in contrast to the very marked decrease within 30 minutes that has been reported in normal full term infants (23)

Since lipolysis is a very insulin sensitive process it was of interest to compare the different insulin responses with the changes in FFA The fall in FFA did not differ significantly in the SGA and AGA infants with the one peak insulin curve versus those with a no response pattern

In the SGA and AGA infants the lack of correlation between the insulin response to an intravenous glucose load and not only the removal rate of glucose but also the decrease of FFA and the fall in $\beta$ hydroxybutyrate concentrations might sugge t a decreased sen

sitivity to insulin in the infants studied This hypothesis is further supported by the finding in premature babies of unchanged or slightly increased blood glucose levels in the presence of a very marked rise in serum insulin after the infusions of amino acids (12)

## SUMMARY

Blood glucose plasma insulin FFA and $\beta$ hydroxybutyrate values during intravenous glucose tolerance were reported in 20 small for gestational age (SGA) and 15 appropriate for gestational age (AGA) low birthweight infants The babies were divided into three groups according to their age when tested <24 hours 24–48 hours and >48 hours

Both the SGA and AGA infants cleared glucose more rapidly with increasing age The change was more marked in the SGA babies The clearance rates were similar to those reported in normal full sized infants

The insulin values before the glucose load were similar in all groups and comparable to those reported in normal newborn infants

The insulin response to glucose was variable There were no significant differences with increasing age or between the two groups of infants The insulin curve of the individual infant followed one of three patterns Most commonly seen was a double peak curve The infants who showed a single peak insulin response had a better but not significantly different glucose tolerance than that of the other babies Infants with no appreciable insulin response still removed glucose from plasma at a rate similar to those with a double peak insulin curve It is concluded that insulin as measured in peripheral plasma could not explain the rate of removal of glucose from the plasma of the newborn low birthweight infant

Infants of low birthweight had higher plasma FFA values as compared to that reported in normal full term infants The FFA values in SGA infants were higher than those in AGA babies In both groups of infants the $\beta$ hydroxybutyrate values were comparable to those reported in normal full term babies Thus there was an unexpected discrepancy between the high FFA and relatively low $\beta$ hydroxybutyrate levels in plasma The fall in plasma FFA and $\beta$ hydroxybutyrate after glucose was minimal but similar in both groups of infants

The finding are compatible with a decreased sensitivity to insulin in the infants studied

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# THE ACID BASE AND ELECTROLYTE PATTERN IN THE ERYTHROBLASTOTIC INFANT AND ITS RELATIONSHIP TO VARIOUS PARAMETERS DURING EXCHANGE TRANSFUSION

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Although the facility with which the newborn infant usually seems to withstand an exchange transfu ion (ET) is very impressive indeed the procedure carries a certain risk The hazard of using blood stored in acid-citrate-dextrose (ACD) for this purpose with the notoriously low pH of this blood has been weighed against the advantage with re pect to storing proper ties Symptoms attributed to the use of ACD blood have included mal-defined entities as hy porxic spells retching shock arrhythmias and circulatory failure (1 8 14 16) The mortality rate varies a great deal notably with the condition of the infant and the reported figures vary from 0 to 7 depending on the selection of the material (5 24) Occurring complications have been considered attribut able to hyperkalemia acidosis circulatory overload hypothermia and hypocalcemia but a definite link between fatality and these condi tions has as yet not been established (8 9 12 27)

From the clinical point of view the use of ACD blood is associated with two major draw backs its low pH value with concomitant ele vation of plasma potassium and the reduced level of ionized calcium both of which may potentially endanger the infant during the pro cedure In fact, these shortcomings of ACD blood have by many been considered strong enough to warrant the use of heparinized blood (3 17 23) or alternatively ACD blood con verted with respect to acidity prior to the ET (2) or the prophylactic treatment of the patient with bicarbonate (4)

Although the reason for these suggestions is clearly that the use of ACD blood for ET pur pose is capable of inducing an acidotic condi tion in the treated child as well as electrolyte disturbances the literature reports with re spect to such changes are very divergent Whereas some authors have reported the pres ence of advanced acidosis in connection with ACD blood (2 4 7 18 24) others have failed to note this (10) and in some instances pH has been reported to increase during the pro cedure (2) The disparity of opinions in the cited literature suggest the existence of para meters not sufficiently evaluated and standard ized

The following investigation was undertaken in order to evaluate the possible influence on electrolyte and pH homeostasis of factors per taining to characteristics of the donor blood of the recipient infant and of the transfusion itself

## MATERIAL AND METHODS

37 newborn infants ranging in birth weight from 1200 to 4800 g (m an 2450) were consecutively in vestigated during their first therapeutic ET Indica tion for the procedure was hyperbilirubinemia in 34 of the cases and in the remaining 3 cases this con

This investigation was supported by the Swedish Na tional Association against Heart and Chest Diseases

Table 1 *Statistical analysis of the effect of exchange transfusion on acid base and electrolyte homeostas*
Numbers denote mean values for the total material in each group Statistical evaluation of differences is based on individual paired observations

| | Before ET n = 65 | Significance of difference | After ET n = 65 | Significance of difference | Recovery n = 37 |
|---|---|---|---|---|---|
| pH | 7 39 | *** | 7 34 | | 7 42 |
| StBi | 21 mEq/l | | 19 mEq/l | * | 22 mEq l |
| K | 4 3 mEq/l | * | 4 0 mEq/l | | 3 5 mEq l |
| Na | 138 mEq/l | — | 137 mEq/l | — | 137 mEq l |
| Ca | 4 5 mEq/l | — | 4 6 mEq/l | — | 4 7 mEq/l |
| $PO_4$ | 6 8 mg/100 ml | — | 6 9 mg/100 ml | — | 7 0 mg/100 ml |

** 0 001 < $p$ < 0 01 $p$ < 0 001

dition was anticipated The cause for hyperbilirubin emia was Rh immunization in 9 ABO immunization in 9 and not serologically explained in the remaining 19 cases The procedure was performed at an aver age infant age of 72 hours (range 3–100 hours)

ACD blood stored not longer than 72 hours was used in all instances In order to increase the hema tocrit of the ACD blood 170 ml of plasma was withdrawn from the 500 ml donor bottle The blood was heated in waterbath to 37 before it reached the infant circulation Body temperature was maintained by means of a thermostatically controlled heating pad

Prior to an ET samples were withdrawn from the well agitated donor bottle and from the infant for analyses Infant blood was taken through the ex change catheter with its tip lodged 5–8 cm inside the umbilical skinfolds This depth has in repeated controls in postmortal specimens been shown to be consistent with the portal vascular region Care was taken to flush the catheter free of donor blood before sampling during an ET The patients were mostly calm or asleep during the procedure and in no in stance were there signs of impaired cardiopulmonary function

25 of the 37 ETs were performed uninterrupted with sampling from the patient before halfway through and at the end of the procedure An addi tional recovery sample was taken 20 minutes after the completed ET Although this technique permits the evaluation of the effect of small as well as big exchanges the recovery sample could only be re lated to the whole exchange In order to study re covery after smaller amounts the remaining 12 in fants were exchanged in two stages with a 20 minute recovery sample secured before the latter part of the ET was started This latter part was subsequently not included in the study In this way a total of 67 sets of observations were recorded with the exchanged amount varying between 50 and 250 ml/kg BW The rate of transfusion which was kept as constant as possible during a given ET or part of it varied from 5 to 30 ml/min between different sets of ob servations

The studied parameters may conveniently be di vided into dependent and non-dependent ones The following dependent variables were investigated pH Pco standard bicarbonate potassium sodium cal cium and phosphorus in the infant's blood Non dependent variables were related to the quality of the donor blood (see pH potassium sodium calcium and phosphorus) to the mode of exchange (ml/kg ml/kg/min) and to patient characteristics (weight age bilirubin level preoperative pH Pco standard bicarbonate potassium sodium calcium and phos phorus)

pH Pco and standard bicarbonate were deter mined by the Astrup micro technique (20) with tem perature correction according to Rosenthal (19) Plasma potassium and sodium were analysed spectro photometrically and calcium and phosphate were as sessed according to standard procedures The statis tical evaluation of the results was carried out on an IBM 7044 computer (Correlation with Transerver ation Health Science Computing Facilities U C L A 1966)

## RESULTS

*Evaluation of dependent variables Acid base changes* There was a significant fall in pH and standard bicarbonate during ET The pre operative values for pH and bicarbonate were completely regained after the 20 minutes of recovery and exceeded them in a few instances (Table 1)

*Electrolyte pattern* Although the effect on the plasma potassium level varied a great deal there was a statistically significant fall in the parameter observed at the end of the proce dure a fall which was further accentuated dur ing the 20 minute recovery period

Table 2 *Acid base and electrolyte characteristics of donor blood*

| Age hr | pH | StBi mEq l | K mEq/l | Ca mEq l | Na mEq l | PO mg/100 ml | Htc % |
|---|---|---|---|---|---|---|---|
| 34±23 | 6 75±0 07 | 5 70±1 68 | 6 21±1 84 | 3 91±0 20 | 143±5 4 | 2 81±1 09 | 54±5 6 |

There was usually a decrease in the level of total calcium during the procedure but the effect on this parameter was very unpredictable and there was overall no statistically significant difference induced during ET or during the recovery period Minor in onsistent changes occurred with respect to sodium and phosphorus

*Evaluation of non-dependent variables* The pre and postoperative characteristics of the infants and of the donor blood are listed in Tables 1 and 2 Although there was a moderate metabolic acidosis present in most of the patients pH was with few exceptions maintained above 7 37 Except for the calcium level which was moderately reduced in a few cases the electrolyte pattern was unremarkable

The acidity of the donor blood was low with minor variations around 6 7 A moderate reduction of total calcium with a slight elevation of the potassium level gave the characteristic pattern of the donor blood

*Relationships between dependent and non-dependent variables* Apart from a highly significant correlation between the volume of ET and decrease in the potassium level of the infant blood och highly significant correlation between the rate of transfusion and the drop in pH and standard bicarbonate (Tabel 3) a significant correlation existed between the preoperative bilirubin level and the potassium decrease during recovery It is particularly noticeable that there exists no correlation between characteristics of the donor blood and changes induced in electrolyte homeostasis

Although the level of Na Ca and $PO_4$ after ET were not significantly different from the preoperative value (Table 1) the magnitude

Table 3 *Overall correlation results Non dependent versus dependent variables*

| | | Patient | | | | | | | | | | Donor | | | | | | | | ET | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BW | Age | Bilir | pH | StBi | K | Ca | Htc | Na | PO | Age | Bilir | pH | K | Ca | Htc | Na | PO | ml/kg | ml/kg /min |
| pH | E | | | | | | | | | | | | | | | | | | | | |
| | R | | | | | | | | | | | | | | | | | | | | |
| StBi | E | | | | | | | | | | | | | | | | | | | | |
| | R | | | | | | | | | | | | | | | | | | | | |
| K | E | | | | | | | | | | | | | | | | | | | | |
| | R | | | | | | | | | | | | | | | | | | | | |
| Ca | E | | | | | | | | | | | | | | | | | | | | |
| | R | | | | | | | | | | | | | | | | | | | | |
| Na | E | | | | | | | | | | | | | | | | | | | | |
| | R | | | | | | | | | | | | | | | | | | | | |
| PO | E | | | | | | | | | | | | | | | | | | | | |
| | R | | | | | | | | | | | | | | | | | | | | |

E change during ET $0.001 < p < 0.01$ R = change during recovery $p < 0.001$

Table 1 *Statistical analysis of the effect of exchange transfusion on acid base and electrolyte homeost...*
Numbers denote mean values for the total material in each group Statistical evaluation of differences is based on individual paired observations

| | Before ET $n=65$ | Significance of difference | After ET $n=65$ | Significance of difference | Recovery $n=37$ |
|---|---|---|---|---|---|
| pH | 7 39 | * | 7 34 | * * | 7 42 |
| StBi | 21 mEq/l | ** | 19 mEq/l | | 22 mEq/l |
| K | 4 3 mEq/l | | 4 0 mEq/l | * * | 3 5 mEq/l |
| Na | 138 mEq/l | — | 137 mEq/l | — | 137 mEq/l |
| Ca | 4 5 mEq/l | — | 4 6 mEq/l | — | 4 7 mEq/l |
| $PO_4$ | 6 8 mg/100 ml | — | 6 9 mg/100 ml | — | 7 0 mg/100 ml |

0 001 < $p$ 0 01 $p$ 0 001

dition was anticipated The cause for hyperbilirubinemia was Rh immunization in 9 ABO immunization in 9 and not serologically explained in the remaining 19 cases The procedure was performed at an average infant age of 72 hours (range 3–100 hours)

ACD blood stored not longer than 72 hours was used in all instances In order to increase the hematocrit of the ACD blood 120 ml of plasma was withdrawn from the 500 ml donor bottle The blood was heated in waterbath to 37 before it reached the infant circulation Body temperature was maintained by means of a thermostatically controlled heating pad

Prior to an ET samples were withdrawn from the well agitated donor bottle and from the infant for analyses Infant blood was taken through the exchange catheter with its tip lodged 5–8 cm inside the umbilical skinfolds This depth has in repeated controls in postmortal specimens been shown to be consistent with the portal vascular region Care was taken to flush the catheter free of donor blood before sampling during an ET The patients were mostly calm or asleep during the procedure and in no instance were there signs of impaired cardiopulmonary function

25 of the 37 ETs were performed uninterrupted with sampling from the patient before halfway through and at the end of the procedure An additional recovery sample was taken 20 minutes after the completed ET Although this technique permits the evaluation of the effect of small as well as big exchanges the recovery sample could only be related to the whole exchange In order to study recovery after smaller amounts the remaining 12 infants were exchanged in two stages with a 20 minute recovery sample secured before the latter part of the ET was started This latter part was subsequently not included in the study In this way a total of 67 sets of observations were recorded with the exchanged amount varying between 50 and 250 ml kg BW The rate of transfusion which was kept as constant as possible during a given ET or part of it varied from 5 to 30 ml/min between different sets of observations

The studied parameters may consequently be divided into dependent and non-dependent ones The following dependent variables were investigated pH Pco standard bicarbonate potassium sodium calcium and phosphorus in the infant's blood Non-dependent variables were related to the quality of the donor blood (age pH potassium sodium calcium and phosphorus) to the mode of exchange (ml/kg ml/kg/min) and to patient characteristics (weight age bilirubin level preoperative pH Pco standard bicarbonate potassium sodium calcium and phosphorus)

pH Pco and standard bicarbonate were determined by the Astrup micro technique (20) with temperature correction according to Rosenthal (19 Plasma potassium and sodium were analysed spectrophotometrically and calcium and phosphate were assessed according to standard procedures The statistical evaluation of the results was carried out on an IBM 7044 computer (Correlation with Transgeneration Health Science Computing Facilities UCLA 1966)

## RESULTS

*Evaluation of dependent variables Acid base changes* There was a significant fall in pH and standard bicarbonate during ET The preoperative values for pH and bicarbonate were completely regained after the 20 minutes of recovery and exceeded them in a few instances (Table 1)

*Electrolyte pattern* Although the effect on the plasma potassium level varied a great deal there was a statistically significant fall in this parameter observed at the end of the procedure a fall which was further accentuated during the 20 minute recovery period

furthermore been pointed out that the removal rate of bilirubin is not relat d to the rate of exchange but rather to the total volume exchanged (22) it is difficult to see any reasons for the routine use of high rate transfusion If for one reason or another a high rate is indicated such as advanced cardiac failure the use of converted ACD blood or the prophylactic use of bicarbonate in the beginning of the procedure should be advocated

In accordance with earlier findings (8) the total plasma calcium remained more or less constant during the whole procedure Possible effects on the ionized fraction could not be evaluated as we considered our means of assessing this fraction over the total protein less reliable

Whereas the expected electrolyte change in an infant receiving ACD blood with its elevated potassium level and low pH would be a state of hyperpotassemia the present findings denote the opposite effect Minor and inconsist nt changes of the plasma potassium have earlier been reported in connection with exchange transfusions (6 8 14) It has been stated (8 14) that the use of ACD blood for ET induces an elevation of infant plasma potassium when donor blood with high potassium level is used whereas there usually is no change with blood of shorter storage time and moderately increased plasma potassium It has been proposed (8) that the probable fate of the infused excess potassium is rapid incorporation in the glycogene is of the liver The present finding that the potassium level is inversely related to the exchanged amount with a further decrease during the recovery period implies the action of a time dependent factor The subnormal (11) plasma potassium values of the recovery samples may be related to the late alkalizing effect of citric acid metabolism but the presence of a highly significant correlation between infant bilirubin level and the depression of plasma potassium during recovery may also imply an interaction with the post-exchange bilirubin rebound phenomenon


## SUMMARY

The effect of exchange transfusion in the electrolyte and acid base balance has been studied in 37 newborn infants undergoing the procedure because of hyperbilirubinemia. Particular interest was focused on possible relationships between induced changes and various pertinent parameters such as infant and donor blood characteristics and rate and volume of the exchange A statistically highly significant decrease in pH and plasma bicarbonate was noted together with a decrease of the potassium level Changes in other electrolytes were inconsistent There was a highly significant correlation between rate of exchange and acid base disturbance as well as between induced potassium decrease and the total volume of the procedure There was no demonstrable increase of the susceptibility to induced changes among the low weight infants A recovery period of 20 minutes was usually sufficient to allow for the control of the metabolic derangement and in some instances an alkalotic over shoot was encountered as related to the pre operative control value The potassium level continued to fall during the recovery period

The technical aspects of the procedure are discussed against the reported findings


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of the induced change was in the individual case highly significantly correlated to the corresponding preoperative value (Table 3)

As a higher susceptibility to induced changes might be expected in the low weight infants they were tested relative to rate and amount against the infant's weight and age Neither of these relationships however showed statistical significance

## DISCUSSION

The present investigation has shown that the use of ACD blood for exchange transfusions results in a usually moderate metabolic acidosis in the patient and that this derangement is mostly affected by the rate of transfusion It is also suggested that factors like weight age pre existing moderate acidosis and severity of the hemolytic disease play a minor role in this respect

Although the finding that the rate of ET bears a close relationship to the induced metabolic acidosis is not surprising in itself the disparity in this respect between rate and volume of ET deserves a few comments

An exchange transfusion is most effective in the beginning of the procedure i e more infant blood is exchanged against donor blood per stroke and consequently the net addition of ACD blood will be relatively smaller as the ET proceeds As not only the pH but also the buffering capacity of the blood seems relatively less affected during the latter part of an ET it is suggested that a steady state with respect to on the one hand net addition of citrate and on the other the metabolic breakdown and acid excretion has been reached It is known that whereas infused citrate rapidly disappears from the blood stream (12) its metabolic breakdown is not an instantaneous process and usually results in a late but longstanding alkalosis (2 7) The load on the buffering system of the blood may consequently be expected to be successively smaller as the ET proceeds This seems in good agreement with the finding that the metabolic acidosis is less pronounced in the latter part of an ET and at the end of the recovery period may even be less than the preoperative value These findings of a late alkalizing effect is in good agreement with earlier reports (2 4 21 25)

The fact that the premature infant has a less well developed renal clearance of hydrogen ions (13) but nevertheless seems equally capable of controlling the induced metabolic acidosis suggests that the citric acid metabolism may play a greater role in the maintenance of pH homeostasis than the acidifying capacity of the kidneys The role played by the kidneys and lungs in the control of the metabolic acidosis during ET has however not been duly evaluated and investigations along that line are presently being initiated in our laboratory

The close relationship observed between rate of ET and the metabolic derangement induced in the infant brings up the question how an exchange transfusion ideally ought to be performed Although there seems to be unanimous agreement that the exchanged amount ought to correspond to approximately twice the estimated blood volume of the infant opinions regarding the rate of the exchange vary considerably

Whereas Mollison (15) advocated that the rate of ET should not exceed an amount corresponding to 250 mg citrate per kg BW an hour (corresponding to 90 ml ACD blood) others have failed to observe adverse effects with considerably higher rates (5 26 27) In clinical routine work the practice most often is probably to let the rate be dependent on the ease with which the blood is withdrawn provided the infant does not show symptoms of ill effects Although we did not encounter any side effects in our patients submitted to high rate transfusion it should be pointed out that while not causing any symptoms in the child in good clinical condition the induced metabolic acidosis must always be appreciated as a potential danger especially in the ill infant, where cardio-vascular derangement may more easily be precipitated With this background and in view of the fact that it has

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# THE VENOUS PLASMA FREE AMINO ACID LEVELS OF MOTHER AND CHILD DURING DELIVERY III

## *In a Lower Socio-economic Group of a Refugee Area in Karachi West Pakistan with Special Reference to the Small for Dates Syndrome*

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Protein undernutrition (low intake of dietary nitrogen) in the pregnant woman might be of importance to foetal development Interference with protein synthetic mechanisms at an early stage of development may not become manifest until much later in life (21) Thus the critical period for initiation of optimal growth has been found to be the suckling period in the rat (48) Restriction of quantity or quality of protein administration to the pregnant and lactating rat leads in the offspring to permanent stunting of growth (8 17) impairment of food utilization delayed neurological development ammonaciduria and deranged kidney development (22 34 51)

The developmental pattern of enzyme activity or enzyme induction shows great variation from species to species This has been demonstrated in the case of the urea synthesizing system where increasing activity is seen after birth in the rat but where high activity is seen during early gestation in the human foetus (32) It could be that a critical period for initiation of optimal growth in the human is during foetal life The maternal regulation of foetal growth seems to be very marked and to obscure genetic differences (43) The effect of food deprivation in the pregnant sheep on functional cotyledon weights (14) and body and liver weight of the litter at birth (42) is considerable in contrast to what has been found in the rat (7 25) and sow (31) There are indications in human studies where there has been an attempt to estimate length of gestation that foetal growth may be retarded by lack of proper maternal diet (2 15 30 35) and there is a striking correlation between paediatric rating of newborns and the maternal level of protein intake (11) The importance of this to the functional adaptation of the newborn in the economically under-developed countries is not clear It seems evident that studies on the human are necessary in order to translate the more extensive knowledge derived from animal experiments

There is a lack of criteria for protein undernutrition (low intake of dietary nitrogen) in the pregnant woman Assessment of protein nutrition by weight increase and urea excretion (50) is bedevilled by pregnancy toxaemia and serum albumen is low during normal pregnancy The extent of hydroxyproline excretion

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Table 1. *Lower socio-economic group (A). Free amino acid levels in venous plasma from the mother during delivery and the cord vein during delivery with ratios in between the cord vein level and that of the mother's cubital vein*

Range, mean and standard deviation are expressed in µmol/l plasma. n = number of cases where the actual amino acid was separated in such a way that an accurate estimation was possible

| | Mothers | | | | Cord | | | | Ratio cord/maternal plasma | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amino acid | Range | n | Mean | S.D. | Range | n | Mean | S.D. | Range | n | Mean | S.D. |
| α alanine | 302–606 | 9 | 416 | 96 | 355–777 | 9 | 551 | 146 | 0.7–1.8 | 8 | 1.4 | 0.4 |
| Glycine | 134–232 | 9 | 178 | 34 | 230–388 | 9 | 370 | 57 | 1.4–2.2 | 8 | 1.8 | 0.3 |
| Valine | 81–169 | 9 | 130 | 31 | 161–284 | 8 | 209 | 38 | 1.3–2.3 | 7 | 1.7 | 0.4 |
| Proline | 43–229 | 9 | 157 | 62 | 143–256 | 9 | 210 | 36 | 1.0–3.9 | 8 | 1.7 | 0.9 |
| Lysine | 88–180 | 9 | 141 | 33 | 276–414 | 9 | 339 | 45 | 1.9–3.5 | 8 | 2.6 | 0.5 |
| Leucine | 47–147 | 9 | 96 | 30 | 102–160 | 9 | [illegible] | 18 | 0.9–2.4 | 8 | 1.5 | 0.5 |
| Taurine | 76–177 | 9 | 109 | 32 | 140–323 | 9 | 223 | 63 | 1.4–3.0 | 8 | 2.0 | 0.5 |
| Arginine | 7–78 | 9 | 39 | 24 | 21–87 | 9 | 53 | 24 | 0.5–10.3 | 8 | 2.4 | 3.3 |
| Histidine | 78–132 | 9 | 104 | 20 | 131–185 | 9 | 150 | 19 | 1.2–1.9 | 8 | 1.4 | 0.3 |
| Isoleucine | 17–48 | 9 | 49 | 17 | 52–81 | 9 | 66 | 10 | 0.8–3.1 | 8 | 1.6 | 0.7 |
| Phenylalanine | 38–82 | 9 | 55 | 16 | 66–105 | 9 | 79 | 13 | 0.8–2.2 | 8 | 1.6 | 0.5 |
| Ornithine | 41–96 | 9 | 68 | 18 | 123–234 | 9 | 180 | 54 | 1.4–3.7 | 8 | 2.7 | 0.8 |
| Tyrosine | 27–53 | 9 | 37 | 10 | 50–89 | 9 | 63 | 12 | 1.3–2.6 | 8 | 1.8 | 0.4 |
| Methionine | 10–22 | 9 | 15 | 4 | 17–37 | 9 | 26 | 30 | 0.9–3.7 | 8 | 1.9 | 0.9 |
| Citrulline | 8–24 | 9 | 15 | 7 | 10–[illegible] | 8 | 17 | 5 | 0.5–1.6 | 7 | 1.1 | 0.4 |
| α-$NH_2$-Bu | 3–16 | 7 | 9 | 5 | 7–9 | 7 | 13 | 8 | 0.5–3.3 | 4 | 1.6 | 1.2 |
| Urea | 1230–4360 | 9 | 2490 | 970 | 1330–4370 | 9 | 2830 | 841 | 0.9–1.3 | 8 | 1.1 | 0.2 |

upon general examination and no complications during the 3-day postnatal stay in the wards. The placentas were normal upon gross examination by one of us immediately after delivery. The mothers had negative malaria smear at the time of delivery and showed no obvious liver disease upon clinical examination. Gross anaemia (Hb < 10 g%) was present in one case in group A. The mothers showed no clinical deficiency symptoms such as angular cheilosis, pre-tibial oedema, goitre, bone pains or signs suggestive of beri beri (18). Most mothers in group A showed weakness and conjunctival pallor and were thin with sometimes marked depletion of subcutaneous fat.

The diet of the group A mothers was mostly vegetables and dal (pulses and lentils). Dal can be compared to dried peas and beans (350 cal/100 g same as cereals, 1.2 g % fat or negligible, 20–25 % protein, twice as much as cereals—compared to animal protein relatively rich in lysine and poor in methionine, high iron (8–10 mg %) and calcium (100–200 mg%) content, a fair source of thiamine, riboflavin, nicotinic acid and vitamins A and C when germinated through soaking in water for a couple of days). Dal forms a rich source of proteins easily digestible if cooked well. One cup of thick dal gives 14 g protein and 200 calories (1). The average helping is one plate twice a day. The habit of eating wheat (chappati) with pulses provides protein of a high biological value (29). People in this area also consume goat or buffalo butter milk (lassi) which might compensate for the otherwise low fat diet. Beef o mutton is consumed once a 1 ice week and fish, rice, egg, fruit and milk rarely.

The mothers of group B had a balanced diet with cereals, dal, rice, vegetables, fruit, egg and meat daily.

The birth weights and lengths are compared to the Swedish standard in Fig. 1. The 10 cases of group A showed weight and length < −2 S.D. in 3 and weight ≤ −2 S.D. in additional 2 cases, while the rest showed length and weight around −1 S.D. The 10 cases of group B had normal lengths, while the weights were around −1 S.D. In order to indicate that the material of group A is representative of the small for dates syndrome (lower birth weight than expected according to gestational age), Fig. 2 gives the birth weights and lengths as compared to the Swedish standard in 34 normal deliveries from the refug e area of group A and 17 normal deliveries from the private hospitals of group B.

Group B is not considered to be a "normal" group but a group in between group A and a not investigated group from a higher Pakistani socio-economical level with a dietary standard comparable to the Swedish mean.

## RESULTS

The results of the plasma ion exchange chromatography are given in Tables 1, 2 and 3. The comparative statistical analysis of the data dealt with 18 variables (16 free amino acid levels, the glycine/valine quotient and urea level) in 12 groups (mother's plasma, cord

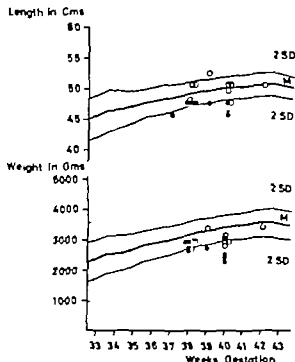



*Fig 1* The 10 investigated cases of the lower socio economical group A (•——•) and of the local reference group B (o——o) The individual birth weights and crown heel lengths have for the sake of simplicity been plotted onto the Swedish standard for girls (13)

(47 49) might be disturbed in pregnancy toxaemia

Protein deficiency in the clinical syndromes of kwashiorkor and marasmus (4 12 16 33 44 45) and experiments with low nitrogen intake (3 37 39) lead to typical changes in the plasma homeostasis of free amino acids in the human It is the aim of the present study to investigate if this characteristic is also valid for the pregnant woman and the foetus where the plasma amino acid homeostasis is changed already during normal conditions (23) The changes seen in short gestation and hypertensive disorder of pregnancy have been investigated earlier (24)

## METHODS

The methods including collection of samples deproteinization and ion-exchange chromatography were identical to those described earlier (23) Because plasma was stored at −20 C until deproteinization around 1–5 months later glutamic acid and aspartic acid were excluded from the assay as their concentrations in plasma increase through deamidation of their amides upon prolonged storage (10)

## MATERIAL

The investigated cases of a lower socio-economic group (A) consisted of 10 women They lived in a refugee area of Karachi West Pakistan and were delivered at the Obstetrical Department of Jinnah Post graduate Medical Centre The family income was 60–150 Rupees (US $12–30) mean 120 Rupees ($22) per month The investigated cases from a middle class group (B) consisted of 10 women delivered at two private hospitals in Karachi (Holy Family and Seventh Day Adventists Hospitals) The family income was 250–1500 Rupees ($50–300) mean 700 Rupees ($140) per month

The mother s mean weight immediately after delivery was 46 kg (height 155 cm) in group A against 58 kg (height 159 cm) in group B Ethnic origin of Bengali Punjabi and Sindhi was evenly distributed between group A and B while there was an over representation of the United Provinces in group A and Bombay origin in group B Age varied between 17–24 years in primigravidae (8 cases, 5 in group A and 3 in group B) and 19–35 years in multiparae (II IX pregnancies 12 cases) Maternal care during pregnancy was negligible in both groups Length of pregnancy was ≥37 weeks All had normal crown vaginal deliveries without cord obstruction and with an Apgar s score of 10 at 1 and 4 minutes after birth No anaesthesia was used The mothers had a blood pressure below 140/90 mm Hg and showed no glucosuria or proteinuria at the time of delivery The babies showed no malformations

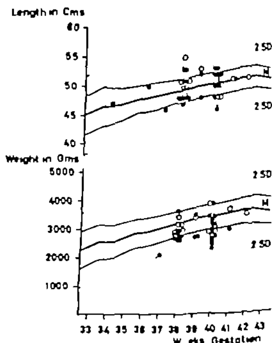



*Fig 2* Birth weights and crown–heel lengths in 33 cases from the refugee area of group A (•——•) and 17 cases from the private hospitals of group B (o——o) for the sake of simplicity plotted onto the Swedish standard for girls (13)

The cord/maternal quotient showed a general tendency towards decrease with the exception of the alanine and urea quotients The ratio was significantly decreased in the case of *ornithine* (**) in the low socio-economical group (A) as compared to the local reference group (B) because of the dominant increase of the maternal level

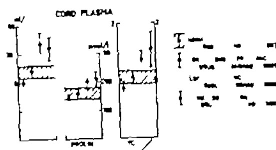


*Fig 4* Free glycine and proline levels and the glycine/valine quotient of cord venous plasma during delivery after normal (23) and short pregnancy (24) in the lower socio-economical group (A) and the local reference group (B)

## DISCUSSION

*Material*

The lower socio-economic group is nutritionally characterized by daily low consumption of vegetable protein during the prematernal stage as well as the actual pregnancy The dietetical survey and the predominance of the calorie deficiency syndrome among children of this area who share the same food habits speaks for a low calorie nutrition in the maternal There were no clinical signs of iodine calcium or vitamin deficiency and gross anaemia was rare A dietetical study of pregnant women in a low socio-economical group (US $20 per month) in India with similar food habits (41) gave an estimate of 38 g protein 1408 cal 315 mg Ca 18 mg iron and 912 IU vitamin A per day with no gross evidence of undernutrition This is to be compared to the recommended minimum protein intake during pregnancy of 75 g per day (6 28)

In spite of the limited material and a lack of local standards of birth weight and length in relation to duration of pregnancy some indications concerning the old problem of birth weight and maternal nutrition can be derived from Fig 2 There is a surprisingly small difference in birth length from the Swedish standard in the middle-class group while the birth weights seem to be about 400 g lower at the 40-week level The middle-class group showed no baby to be ≤ −2 SD in length or weight while one baby was over +2 SD long This is in contrast to the strikingly short stature and low weight of the adults of this racial and socio-economical group In the lower socio-economic group there are 18 of babies with a birth weight ≤ −2 SD of whom /₃ also show a length ≤ −2 SD Fig 2 indicates that these babies would show low weight and length even in relation to a hypothetical lower local standard due to racial differences Birth weight studies in a larger sample (1100 cases) of the same socio-economic group in Karachi have shown 17 of weight below 2500 g in spite of gestational age ≥ 37 weeks (30)

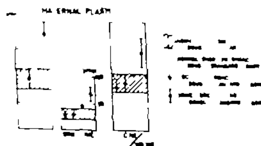


*Fig 3* Free glycine and ornithine levels and the glycine/valine quotient of maternal venous plasma during delivery after normal (23) and short pregnancy (24) in the lower socioeconomical group (A) and the local reference group (B)

These or similar antropometric findings in studies of birth weight reduction of lower socio-economic groups cannot be interpreted as an effect of maternal nutrition alone as other external factors can influence foetal growth Manual labour during pregnancy is more common in these groups This might explain the greater birth weight reduction found by Antonov (2) during the siege of Leningrad where women had to work extremely hard in addition

Table 2 *Reference group (B)*
For explanation see Table 1

| Amino acid | Mothers Range | *n* | Mean | S D | Cord Range | *n* | Mean | S D | Ratio cord/maternal plasma Range | *n* | Mean | S D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| α alanine | 294–463 | 9 | 366 | 51 | 390 674 | 9 | 500 | 84 | 1 0–2 0 | 8 | 1 4 | 0 3 |
| Glycine | 116–210 | 9 | 165 | 29 | 242–433 | 9 | 334 | 58 | 1 4–2 7 | 8 | 1 9 | 0 4 |
| Valine | 115–190 | 9 | 148 | 23 | 210 294 | 9 | 243 | 28 | 1 4 2 0 | 8 | 1 7 | 0 ? |
| Proline | 113–273 | 9 | 161 | 49 | 166–261 | 9 | 200 | 37 | 1 0 1 9 | 8 | 1 3 | 0 3 |
| Lysine | 109 173 | 9 | 141 | 23 | 258–501 | 9 | 367 | 73 | 2 0–3 7 | 8 | 2 6 | 0 6 |
| Leucine | 72–125 | 9 | 101 | 21 | 96–183 | 9 | 137 | 32 | 1 0–1 6 | 8 | 1 3 | 0 ? |
| Taurine | 54–245 | 9 | 138 | 65 | 151–446 | 9 | 247 | 91 | 1 3 3 7 | 8 | 2 0 | 0 9 |
| Arginine | 53–99 | 9 | 73 | 14 | 36–89 | 8 | 64 | 18 | 0 5 1 3 | 8 | 0 9 | 0 3 |
| Histidine | 61–115 | 9 | 94 | 17 | 116–181 | 9 | 136 | 23 | 1 1–1 7 | 8 | 1 4 | 0 2 |
| Isoleucine | 40 64 | 9 | 54 | 9 | 58–101 | 9 | 75 | 14 | 1 1–1 6 | 8 | 1 4 | 0 ? |
| Phenylalanine | 39 68 | 9 | 55 | 10 | 54–104 | 9 | 80 | 18 | 1 1 1 8 | 8 | 1 5 | 0 ? |
| Ornithine | 28–88 | 9 | 44 | 17 | 120–294 | 9 | 170 | 54 | 3 1–4 4 | 8 | 3 8 | 0 5 |
| Tyrosine | 29–65 | 9 | 42 | 11 | 53–103 | 9 | 69 | 16 | 1 3–1 9 | 8 | 1 6 | 0 ? |
| Methionine | 12–28 | 9 | 18 | 5 | 27–50 | 9 | 36 | 9 | 1 4–3 1 | 8 | 2 1 | 0 6 |
| Citrulline | 4–26 | 9 | 13 | 7 | 11–37 | 8 | 17 | 9 | 1 0 1 4 | 8 | 1 2 | 0 2 |
| α-$NH_2$-Bu | 3–14 | 9 | 9 | 4 | 9–23 | 7 | 16 | 6 | 1 3–3 0 | 7 | 1 9 | 0 6 |
| Urea | 1710–3630 | 9 | 2370 | 559 | 1490–3760 | 9 | 2450 | 712 | 1 0–1 3 | 8 | 1 1 | 0 1 |

plasma the maternal/cord ratio in normal deliveries (23) short gestation (24) and group A and B of the present investigation) Mean ± double standard error for every variable in each group was calculated and whenever group A or B did not show overlapping with the normal and short gestation groups a t test was made to confirm statistical significance of difference

The mothers plasma showed a general tendency towards increased amino acid levels with the exception of the *valine* and *urea* levels The non essential amino acids *glycine* and *ornithine* were significantly increased (*** $p < 0.1$ ) as was the *glycine/valine* quotient (** $0.1 < p < 1$ ) See Fig 3 *Arginine* was significantly decreased (**) in the low socio-economic group (A) as compared to the local reference group (B)

The cord plasma showed a general tendency towards increased amino acid levels with the exception of the *valine leucine isoleucine tyrosine arginine* and *urea* levels The non essential amino acids *glycine* and *proline* were significantly increased (* *) as was the *glycine/valine* quotient (* *) See Fig 4

Table 3 *The glycine/valine quotient in venous plasma from the mother during delivery and the cord vein during delivery*
*n* = number of cases where the two amino acids were separated in such a way that an accurate estimation was possible

| Clinical group | Mothers Range | *n* | Mean | S D | Double standard error | Cord Range | *n* | Mean | S D | Double standard error |
|---|---|---|---|---|---|---|---|---|---|---|
| Normal (23) | 0 49–1 33 | 10 | 0 87 | 0 27 | 0 17 | 0 80–1 39 | 10 | 1 07 | 0 18 | 0 11 |
| Short gestation (24) | 0 53–1 02 | 6 | 0 78 | 0 21 | 0 17 | 0 74–1 09 | 5 | 0 86 | 0 14 | 0 12 |
| Group A | 0 83–2 20 | 9 | 1 44 | 0 41 | 0 27 | 1 00–2 37 | 8 | 1 55 | 0 45 | 0 32 |
| Group B | 0 83–1 42 | 9 | 1 13 | 0 17 | 0 11 | 1 04–1 74 | 9 | 1 38 | 0 4 | 0 16 |
| Hypertensive disorder (24) | 0 61–1 37 | 6 | 0 95 | 0 29 | 0 24 | 0 82–1 67 | 8 | 1 33 | 0 30 | 0 21 |
| Hypertensive disorder + the small for dates syndrome (24) | 0 61–1 12 | 3 | 0 89 | 0 26 | 0 24 | 0 82 1 67 | 4 | 1 40 | 0 39 | 0 39 |

group themselves for transport has been pointed out (9)

The absolute increase of one amino acid (glycine) level alone or of the total amino nitrogen level (50) should not be used as a sampling criterion as (a) it is bedevilled by hyperammonaemia on any other basis (b) there are divergent reports in the literature concerning general hypo- or hyper aminoacidemia in protein undernutrition (3 4 5 12 16) and (c) the maternal plasma glycine level is slightly increased during hypertensive disorder of pregnancy (24) Arginine plasma level is represented in the chromatogram by a low broad peak and therefore difficult to determine in maternal plasma where it normally shows a lowered concentration (23) The possibility of using the ornithine/arginine quotient is therefore not practical The use of a quotient between several non essential and essential amino acids after paper-chromatography (46) has caused controversial results (20 26 45) possibly through the influence of the increased glutamine level (39) and the fact that the plasma glutamine level decreases even upon storage of deproteinized plasma at −20 C (10)

Fig 4 shows that the increased glycine/valine quotient is also seen in cord plasma Short gestation does not disturb the use of the quotient as an index of protein undernutrition during pregnancy (Figs 3 4) There is an increase of the quotient from the short pregnancy group to the ones born at term (Fig 4) In agreement with this finding the glycine level has been found to increase in cord plasma during pregnancy of the Rhesus monkey (19) while the valine level and the cord/maternal ratio for all amino acids decreased with increasing gestational age

Fig 5 shows that the maternal glycine/valine quotient is not changed by hypertension as a complication during pregnancy (24) The cord quotient however seems to be increased in hypertension + the small for dates syndrome


## SUMMARY

The plasma aminograms of mother s and cord vein plasma during delivery were studied in selected cases from a lower socio-economical group in West Pakistan with a high incidence of intrauterine growth retardation of the foetus

There was a highly significant increase in the levels of the *non-essential amino acids* glycine and ornithine in the mother s plasma and of glycine and proline in cord plasma The plasma glycine/valine quotient was significantly increased in maternal as well as in cord plasma

It is suggested that the plasma glycine/valine quotient may serve as an index of subclinical protein undernutrition (low intake of dietary nitrogen) of mother and foetus and thus become a tool in the investigation of the immediate and late effects of this condition during pregnancy in human populations The maternal quotient is not changed by short gestation or hypertensive disorder during pregnancy



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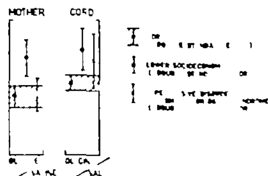


*Fig 5* The glycine/valine quotient of maternal and cord venous plasma during delivery after normal (23) pregnancy in the lower socio-economical group (A) and in hypertensive disorder of pregnancy where the offspring was of low birth weight and crown-heel length for gestational age (24)

to food deprivation during pregnancy than by Smith (35) in his study of the effect of the hunger winter during the siege of Rotterdam Utero placental blood flow has been reported to decrease considerably during manual labour (27) and is one possible cause of a hypothetical deficient transport of nutrients over the utero placental unit The effect of undernutrition on foetal growth is probably even more evident in the case of permanent deficiency during the prematernal as well as pregnant stage of the mother (40) (presumably *via* a hormonal disturbance) than drastic changes during pregnancy alone A combination of low maternal nutrition and a placental transport insufficiency is therefore to be expected

## Results

The only statistically significant changes of *increased non essential amino acid levels* (glycine ornithine proline) of an *increased glycine/valine quotient* and an inverse relationship in between *arginine* and *ornithine* are identical to the changes found by ion exchange chromatography in the clinical syndrome of less severe kwashiorkor (16) The branch chained amino acid levels in the maternal plasma were normal not lowered as in protein deficiency They should be seen in contrast to the general increase of free amino acids and might also be explained by the fact that in the low socio-economic group of the present material we deal with a combination of maternal undernutrition and the small for dates syndrome In one of the maternal conditions associated with the SFD syndrome (pregnancy toxaemia) the maternal levels of valine isoleucine and leucine have been shown to be increased (24) The only amino acids which did not show an increase in cord plasma (valine isoleucine leucine tyrosine and arginine) are identical to the ones decreased in protein deficiency (3 4 16 33 37)

It seems as if the increased plasma *glycine/valine quotient* which has already been shown as a characteristic of protein undernutrition (low intake of dietary nitrogen) in infants (37) and children (3 4 33) could also be a similar characteristic during pregnancy It has been shown in chicks how the proportion of indispensable amino acids/dispensable amino acids is critical for growth if the nitrogen intake is low (38) The remarkably constant findings in the protein deficiency syndrome of different countries upon different diets (16) and in experiments with low protein diets (3 36 37 39) have led to the conclusion that the changed plasma amino acid pattern mirrors a low total nitrogen rather than a low specific amino acid intake (16 36) The increased ornithine/arginine and phenylalanine/tyrosine quotients in *severe* protein deficiency has been proposed to mean that excessive lack of nitrogen affects normal enzyme activity of amino acid metabolism (44)

The amino acids with polar side chains are concentrated more strongly and by different systems into Ehrlich cells than those with bulky branched apolar side chains (e g valine) It might be that in *subclinical* protein deficiency essentiality (e g valine) and non essentiality (e g glycine) is not in itself relevant to the changed plasma levels A hormonal disturbance could affect the different cellular transport systems differently The close correspondence in the way certain plasma amino acid levels change together in malnutrition and in the way the same amino acids

n group themselves for transport has been pointed out (9)

The absolute increase of one amino acid (glycine) level alone or of the total amino nit rogen level (50) should not be used as a sam pling criterion as (a) it is bedevilled by hyper ammonaemia on any other basis (b) there are divergent reports in the literature concern ing general hypo- or hyper ammonaemia in protein undernutrition (3 4 5 12 16) and (c) the maternal plasma glycine level is slightly increased during hypertensive disorder of preg nancy (24) Arginine plasma level is repre sented in the chromatogram by a low broad peak and therefore difficult to determine in maternal plasma where it normally shows a lowered concentration (23) The possibility of using the ornithine/arginine quotient is there fore not practical The use of a quotient be tween several non-essential and essential amino acids after paper-chromatography (46) has caused controversial results (20 26 45) pos sibly through the influence of the increased glutamine level (39) and the fact that the plasma glutamine level decreases even upon storage of deproteinized plasma at −20 C (10)

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Table 1. *Patient material*

| Case no. | Sex | Age in years | No. of infections | O group of *E. coli* isolated during present infection | Serum samples: O group specificity of precipitating antibodies | Serum samples: Days after onset of present attack of pyelonephritis |
|---|---|---|---|---|---|---|
| *A. First attack* | | | | | | |
| 1 | M | 1/12 | 1 | n.g. | | 65 90 |
| 2 | F | 2/12 | 1 | n.g. | | 10 45 75 |
| 3 | M | 4/12 | 1 | O 4 | | 2 67 |
| 4 | M | 4/12 | 1 | O 2 | O 2 | 5 |
| 5 | M | 5/12 | 1 | O 1 | O 1 | 6 54 90 |
| 6 | F | 5/12 | 1 | O 2 | | 60 99 |
| 7 | M | 5/12 | 1 | O 2 | O 1 O 2 O 4 | 60 |
| 8 | F | 7/12 | 1 | n.g. | | 1 87 |
| 9 | M | 8/12 | 1 | O 6 | | 55 |
| 10 | M | 11/12 | 1 | n.g. | | 12 25 |
| 11 | F | 1 4/12 | 1 | O 1 | O 1 | 7 24 52 |
| 12 | F | 1 11/12 | 1 | O 18 | | 9 31 |
| 13 | F | 3 | 1 | O 18 | | 4 17 45 |
| 14 | F | 5 | 1 | O 4 | | 3 32 65 |
| 15 | F | 5 | 1 | O 7 | | 2 40 68 100 |
| 16 | F | 7 | 1 | O 1 | O 1 | 5 45 73 |
| 17 | F | 8 | 1 | O 2 | | 44 100 |
| 18 | F | 9 | 1 | n.g. | | 1 19 |
| 19 | F | 10 | 1 | O 1 | | 9 44 |
| 20 | F | 15 | 1 | n.g. | | 46 |
| *B. Recurrent* | | | | | | |
| 21 | F | 5 | 2 3 | O 18 | O 18 | 120 |
| 22 | F | 5 | 2 | O 1 | O 1 | 1 34 54 94 |
| 23 | F | 5 | 3 | O 2 | O 2 | 3 24 |
| 24 | F | 5 | 3 | n.g. | n.g. | 30 40 80 110 170 |
| 25 | F | 6 | 2 | O 18 | O 18 | 2 40 67 |
| 26 | F | 6 | >5 | O 8 | O 8 | 43 |
| 27 | F | 6 | 4–5 | O 1 | O 1 | 30 |
| 28 | F | 8 | >5 | O 7 | O 6 | 62 |
| 29 | F | 8 | >5 | O 2 | O 2 | 1 3 41 |
| 30 | F | 9 | 3 | O 7 | O 7 | 7 40 61 290 |
| 31 | F | 10 | 5 | O 2 | O 2 O 6 | 3 41 |
| 32 | F | 11 | 4–5 | O 7 | O 6 | 5 55 |
| 33 | F | 11 | 2 | O 4 | O 4 | 11 |

n.g. Not groupable with antisera against O 1, O 2, O 4, O 6, O 7, O 8, O 18, O 75.
Antibodies reacted with O antigen of urine isolate.

21 in children more than two years old), micro-sedimentation rate more than 20 mm/h (23/27) and increased white cell count in the urine, i.e. >50 cells/ml in girls and >25 cells/ml in boys (32/33). The presence and absence of the listed symptoms and signs were evenly divided between the two groups of patients. The age and sex distribution of the patients is seen in Table 1.

In all patients a careful history was taken. Only those without any previous history of symptoms or signs of urinary tract infection or repeated attacks of fever or illness of unknown cause were selected for the material of patients with first attack of pyelonephritis. Of those diagnosed as having recurrent pyelonephritis all had had at least one and usually several earlier attacks of pyelonephritis with positive *E. coli* cultures from the urine (Table 1). All of the 13 patients with recurrences and 11 of the 20 patients with first attack of pyelonephritis were investigated with i.v. urography and urethrocystography. None of them had any signs of obstructive malformation of the urinary tract. All of the patients were given chemotherapy according to the antibiogram of the infecting *E. coli* strain. First and second infections were treated for ten days. The effect of treatment was verified by repeated urinary cultures after termination of therapy. In patients with further infections this short course of chemotherapy was followed by several months of "prophylactic" administration of an antibiotic in reduced doses.

Blood samples from these infants and children were obtained at various intervals during and after the

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# PRECIPITATING ANTIBODIES TO *E COLI* O ANTIGENS A SUGGESTED DIFFERENCE IN THE ANTIBODY RESPONSE OF INFANTS AND CHILDREN WITH FIRST AND RECURRENT ATTACKS OF PYELONEPHRITIS

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Using agglutination and passive haemagglutination methods several authors have shown that pyelonephritis caused by *E coli* elicits a marked antibody response to the infecting organism (2 17 18 21 22 29 30) So far however no differences in the ability to produce agglutinating antibodies to *E coli* O antigen have been demonstrated in patients with differences in the severity of the disease process i e those with only one infection those with recurring infections and those with renal scars (2 30)

Agglutination methods favour antibodies of the IgM type which originally was reported to be the only antibody type formed in response to enterobacterial O antigens (3) From our earlier studies of the antibody response to the O antigen of the infecting *E coli* strain in children with pyelonephritis it was evident however that antibodies with characteristics other than IgM were present as well (7 8) Thus IgG antibodies were found in a few patients with recurrent infections and reduction of renal parenchyma on intravenous urography Similar findings have been reported in adults by Vosti & Remington (23 24) These observations suggested that a correlation between the severity of the disease process and the IgG antibody response might exist. If so this correlation might be used for diagnostic and prognostic evaluation or possibly in studies of the pathogenesis of pyelonephritis

In the present report the occurrence of precipitating antibodies to *E coli* O antigen in two groups of patients—those with first and those with recurrent attacks of pyelonephritis—has been analysed with immunodiffusion techniques Immunoprecipitation methods are known to favour IgG antibodies (19)

## MATERIAL

The patient material which consisted of 33 infants and children with pyelonephritis was selected from patients with symptoms and signs of urinary tract infections The selected patients fulfilled the following two criteria

1 significant *E coli* bacteriuria, i e >100 000 bacteria ml urine
2 increased passive haemagglutination titres against *E coli* O antigen of the infecting strain (2)

They were divided into two groups 20 patients with an apparent first attack and 13 patients with a recurrent attack of pyelonephritis

Most of the patients had a temperature above 38 0 C (29 out of 33) abdominal pain (17 out of

Presented in part at the 10th meeting of the European Club of Paediatric Research Wien 1968 (11)

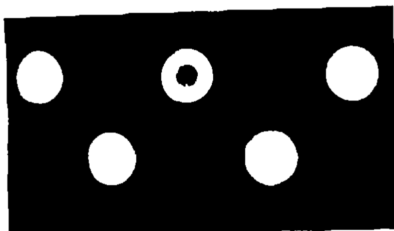


*Fig 1* Double diffusion analysis of three consecutive serum samples from case 22 (three upper basins) with O 1 lipopolysaccharide (two lower basins) showing the presence of precipitating O antibodies in all serum samples

32 22 and 30) the antibodies were no longer demonstrable on investigation 1 1/ 2 3 and 10 months respectively after onset of the actual infection

## DISCUSSION

Agglutinating antibodies against O antigen of the infecting *E coli* organisms regularly increase in titre during the first as well as recurrent attacks of non-obstructive pyelonephritis in infants and children (i a 2 30) In contrast to this the results of the present study suggest that precipitating antibodies to O antigen regularly seem to appear in patients with recurrent attacks of pyelonephritis but not as often during an apparent first infection The validity of this difference is obviously dependent on how correct the clinical allocation has been The recurrent infection group is probably well defined since only patients with at least one previous infection evidenced both by clinical symptoms and by urinary cultures were accepted The *first* episode group is more unreliable since earlier unrecognized infections are difficult to rule out As a matter of fact the finding of antibodies against three different O antigens in one patient in this group (case 7) indicates that this patient might have had two earlier unrecognized infections This interpretation is supported by the observation that cross reactions do not occur between the O antigens of the eight groups employed in this study using precipitation techniques with the single exception of a cross reaction between 04 and 018 (9)

The presence of precipitating antibodies in the patient just mentioned and two other infants (case 4 and 5) in the group with first attack of pyelonephritis is probably not due to the placental transfer of maternal antibodies since the babies were 4–5 months old at the time of sampling and a rather unsensitive antibody detection method was used Furthermore precipitating O antibodies have not been found in sera from healthy infants of similar age or in any of the umbilical cord sera from the healthy infants with healthy mothers (Table 2)

In evaluating the difference in the immune response between the two groups of patients it should be noticed that the materials are not quite comparable as regards age and sex The difference in age presumably is of minor importance since also small infants seem to be capable of producing precipitating antibodies (cf cases 4 5 and 7)

The time of blood sampling after onset of infection is of importance for the detection of the precipitating O antibodies In most instances the first sample was taken early (Fig 2) but occasionally it was taken as late as 6–9 weeks after onset of the infection When precipitating antibodies were found initially they were usually also present in the 6–9 weeks samples

Table 2 *Control material*

| Age group | No | Precipitating antibodies present |
|---|---|---|
| Neonates | 12 | 0 |
| 1–6 mo | 7 | 0 |
| 7–12 mo | 7 | 0 |
| 1–5 y | 8 | 0 |
| 6–10 y | 20 | 0 |
| 11 15 y | 19 | 0 |
| Adults | 20 | 0 |
| Total | 93 | 0 |

actual infection (Table 1) The *E coli* strains isolated from the urine were O grouped with rabbit antisera to the groups 1 2 4 6 7 8 18 and 75 (13)

As controls were used umbilical cord sera from 12 healthy neonates with healthy mothers as well as blood samples from 20 adult blood donors In addition sera from 61 infants and children without any history of urinary tract infections without leucocyturia or bacteriuria and with passive haemagglutination titres within the normal range (2) were included The age distribution is shown in Table 2

## METHODS

*Passive haemagglutination titrations* of the sera of each patient were performed with an O antigen preparation of the *E coli* strain isolated from the patient s urine or of a type strain of the same O group (cf 30) The O antigen was made by boiling a bacterial suspension (density corresponding to McFarland 4) for 2 hours In some cases a pool of O antigens from *E coli* type strains belonging to the eight O groups 1 2 4 6 7 8 18 and 75 most prevalent in patients with urinary tract infections was used as antigen (1)

*The precipitation analyses* were made with a micromodification of the double *diffusion in gel method* (25) All sera excepting those from the neonates were tested with two types of O antigen preparations from the eight *E coli* strains listed above

1 Supernates after centrifugation of boiled bacterial suspensions were employed i e preparations similar to the antigen preparations used for the passive haemagglutination titrations but at much higher concentrations (density of the bacterial suspensions corresponding to 50 mg acetone dried bacteria/ml) Such O antigen preparations contain several precipitinogenic factors in addition to the O antigen (10)

2 O antigens of the eight O groups mentioned above purified according to Westphal *et al* (27) and kindly supplied by Drs B and K Jann Max Planck Institut fur Immunbiologie Freiburg were used for verification of the O antigen specificity of the demonstrated antibodies These lipolysaccharides were employed in concentrations of 1 mg/ml

The umbilical cord sera were only tested with O antigens from the boiled bacterial suspension In a few patients where the *E coli* strain isolated from the urine did not belong to any of the eight O groups mentioned above (designated n e in Table 1) the supernate of a boiled bacterial suspension of the patient s own strain was also used as antigen for the precipitation studies

## RESULTS

In the 93 control sera no precipitating antibodies were found (Table 2) when they were analysed by means of double diffusion with the purified lipopolysaccharides and boiled suspensions ( O antigens ) from type strains of the eight O groups of *E coli* most prevalent in urinary tract infections

In sera from five of the 20 infants and children with an apparent first attack of pyelonephritis precipitating antibodies to *E coli* O antigen were demonstrable In four of these patients the antibodies were directed only against the O antigen of the same O group specificity as that of their own infecting strain (cases 4 5 11 and 16 in Table 1) The fifth patient (case 7) had precipitating O antibodies against the infecting strain as well as against O antigen from two other *E coli* O groups

Precipitating O antibodies were found in sera from all of the 13 patients with recurrent pyelonephritis The precipitation analysis of consecutive serum samples from case 22 shows a typical pattern in Fig 1 The patients antibodies were mostly directed against the O antigen of the same group as that of the infecting strain (Table 1) In sera from two of the children (cases 28 and 32) however the antibodies were directed against a strain different from the one isolated from the urine during the last infection In case 31 antibodies against two different O antigens were present one being similar to that of the infecting strain

From the diagram of the timing of the serum sampling shown in Fig 2 it appears that precipitating antibodies can be registered as early as a few days after onset of symptoms of pyelonephritis The antibodies are then usually found for the following 2 months and in many cases even longer In four patients (cases 31

Early recognition of the patients with a tendency to develop recurrent infection would offer an opportunity for intensified supervision and treatment and thereby possibly diminish the risk of development of progressive renal scarring. For the moment we are not aware of any method for early identification of the patients with high risk. The fact that the precipitating O antibodies appeared in all our patients with recurrent infections but only in a few with their *first infections* suggests that the demonstration of such antibodies might be helpful in the detection of patients at risk.

Preliminary work shows that a modification of the immunodiffusion method used in this work allows rapid and simple screening for these precipitating antibodies to *E. coli* in large numbers of patient sera. The usefulness of such a screening procedure will have to be tested on a larger patient material *followed longitudinally*.

## SUMMARY

The presence of precipitating antibodies to *E. coli* O antigens in consecutive serum samples from infants and children with pyelonephritis without obstructions in the urinary tract was studied using an immunodiffusion method. Precipitating antibodies were found in only five of 20 patients clinically diagnosed as having their *first* attack of pyelonephritis but in all of 13 patients with a *recurrent* infection. Such antibodies were not found in any of 93 controls without symptoms or signs of urinary tract infection.

The reason for the appearance of IgG precipitins to *E. coli* mainly in patients with recurrent pyelonephritis is discussed with regard to three possibilities: a secondary antibody response induced by the recurrent infection, an *adjuvant effect* of endotoxin remaining from an earlier infection and finally more severe infections causing stronger antigenic stimuli in patients with tendency toward recurrences.

The detection of precipitating antibodies to *E. coli* O antigens might be useful for early recognition of patients with recurrent pyelonephritis who are at risk to develop progressive renal scarring.

## ACKNOWLEDGEMENTS

The skilful technical assistance of Mrs G. Jonasson, Misses B. Berglund and I. Svardstrom is appreciated.

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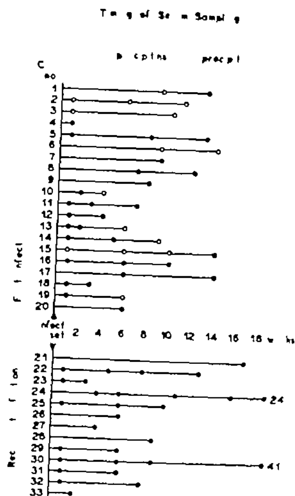


Fig 2 Diagram showing the timing of patient serum samples with or without O precipitins

The reason for the formation of precipitating antibodies mainly in patients with recurrent attacks of pyelonephritis is obscure at present The observed dissimilarities may largely be of a quantitative rather than of a qualitative nature The precipitation techniques may be too insensitive to detect small amounts of precipitating antibodies possibly present already during the first attack of pyelonephritis This assumption is supported by the observation that reduction resistant antibodies can be demonstrated also in some children with their first infection using the passive haemagglutination technique (6)

The precipitating O antibodies were of the IgG type in all patients examined so far (6) Antibodies of this immunoglobulin class generally constitute the major part of the secondary antibody response Therefore the possibility of a booster effect in the patients with recurrent infection seems attractive but is opposed by the observation that in 80 % of the recurrences the bacterial strain is different from that of the preceeding infection (5 15) It should be added however that endotoxin has an adjuvant effect on the antibody response (16) It has also been shown that bacterial endotoxin may persist in the kidney for a long time after experimental acute pyelonephritis (20) Such endotoxin persisting after one infection might function as an adjuvant for the antigens of a different *E coli* strain causing the next infection Since adjuvants seem to increase the 7 S antibody response preferentially (28) such a mechanism might explain the appearance during recurrent infections of high amounts of IgG antibodies demonstrable by precipitation methods

The observed differences in the immune response of the two groups—apparent first infection and recurrent infection—may also be related to clinical dissimilarities Evidence of such dissimilarities is furnished by Bergstrom (4) who found that the risk of getting repeatedly new infections is twice as great in a patient who has already had one recurrence than in a patient with apparent first infection by Lindblad & Ekengren (14) who showed that small girls with early recurrencies have a considerable risk of developing renal scarring and by Leigh *et al* (12) who demonstrated a close correlation in pregnant women between early recurrence and prognosis both with regard to liability to future recurring infections and to renal scarring These findings might indicate a defect in the defense mechanisms of these patients which results in the recurrent infections and possibly also in more severe infections sometimes proceeding to progressive renal scarring The infections in these patients may constitute a stronger antigenic stimulus resulting in IgG production This may be a parallel to the finding in rabbits that repeated or high antigen doses are needed to induce IgG antibody formation against enterobacterial O antigens (26)

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# IMMUNOGLOBULIN LEVELS DURING CHILDHOOD WITH SPECIAL REGARD TO IgE

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In previous papers the concentrations of the immunoglobulins G A M and D have been reported partly from a cross sectional study of healthy children up to the age of 5 years (6) and partly from a longitudinal study of the immunoglobulin development during the first year of life (2) The levels of a fifth immunoglobulin IgE (IgND) in a number of children up to the age of 5 years and also in adults have been reported previously (8) No complete investigation of the immunoglobulin levels in Swedish children of ages 6–15 years has been reported previously and no reports at all have been made of the IgE levels in children older than 5 years

## MATERIAL AND METHODS

The subjects investigated were selected at random by the Central Department of Statistics Stockholm for a study of poliomyelitis immunity in Sweden arranged by the National Bacteriological Laboratory Stockholm The local investigation in Uppsala was carried out under the leadership of one of the authors (T B) this investigation comprised both children and adults Of the 26[illegible] children of 2 15 years of age who were requested to come for the investigation 219 (84 per cent) attended The series included more boys (127) than girls (9[illegible]) which was partly due to the fact that somewhat more boys than girls were called for the investigation and partly to the fact that more boys (88 per cent) than girls (79 per cent) attended

At the time of sample-taking a case history was taken of each child with special respect to various allergic manifestations to past diseases of other kinds and to the general state of health A completely negative history with regard to allergic symptoms and presence of other diseases was found in 138 out of the 219 children investigated Thirtytwo children had or had had symptoms as in atopic diseases (asthma allergic nasal catarrh, flexural eczema urticaria) In a further 28 children, the case history gave reasons to suspect an atopic disposition or at least this could not be excluded The remainder included children with a history of for example deflnate or suspected hypersensitivity to various drugs and transient skin changes with a localization other than flexor surfaces

The results of the immunoglobulin determinations in four children were not included in the statistical analyses Two of these children were given regular gammaglobulin injections for general sensitivity to infection one child had Henoch Schonlein purpura at the time of the investigation and one lacked IgA (IgA < 1 mg/100 ml)

In a further 10 children with no allergic manifestations a longitudinal study of the IgE development was made from the age of 6 weeks to one year These children constitute a part of the paediatric series for which other immunoglobulins have been reported previously (2)

Finally in order to obtain an idea of the IgE development during the first weeks of life capillary samples from six children taken originally for another purpose were tested Since the quantities of serum from each occasion of sample taking were not adequate for IgE analyses, the IgE concentration was determined both in pooled samples from the 2nd–4th days of life and in pooled samples taken when the children were 2 and 3 weeks old

From the 10 children in the longitudinal study and also from the six children mentioned above capillary blood samples were taken while from the remainder in the majority of cases venous samples were taken In some cases the amount of serum obtained from capillary samples was only sufficient for IgE determination The serum was separated after 2 3 hours at room temperature and stored at −20°C until analysed The concentrations of the immunoglobulins G A, M and D were determined by single radial diffusion in agar gel according to the method

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Table 1. *IgE concentrations in ng/ml (arithmetic mean ± S.D., range, geometric mean and confidence limits) in 132 healthy children divided into age groups*

| Age (years) | Healthy children without allergic symptoms | |
|---|---|---|
| | Arithmetic mean ± S.D. (Range) | Geometric mean (95 per cent confidence interval) |
| ?5 | 137 ± 47 (73–185) | 129 (58–286) |
| 3 (2.5–3.5) | 140 ± 58 (64–?80) | 129 (57–294) |
| 4 (3.5–4.5) | 178 ± 93 (67–308) | 154 (48–494) |
| 5–6 (4.5–6.5) | ?09 ± 119 (63–487) | 183 (64–5?3) |
| 7–8 (6.5–8.5) | 251 ± 167 (65–535) | 199 (46–861) |
| 9–10 (8.5–10.5) | ?56 ± 158 (69–530) | 209 (53–8?8) |
| 11–12 (10.5–12.5) | 239 ± 169 (69–715) | 196 (55–703) |
| 13–15 (12.5–15.5) | 330 ± ?12 (54–840) | 268 (69–1040) |

tioned. In the figure regression lines are drawn (log IgE on age) for boys and girls, respectively, as well as the confidence limits.

IgE concentrations in six children lay clearly outside the normal variations for the age and they are not included in the statistical analysis. These children will be discussed later in this paper.

As is evident from Fig. 2 and Table 1, the mean IgE concentrations rose evenly and slowly throughout childhood.

The upper limit value also increased gradually with increasing age, while the lower limit value remained on the whole at an unchanged low level. A regression analysis with regard to age and sex showed a statistically significant relationship between age and IgE level ($p <$ 0.001), while there appeared to be no sex difference with respect to this immunoglobulin.

In Tables 2–5 the concentrations of the other four immunoglobulins G, A, M and D are given both for 132 children with no allergic manifestations or raised IgE values, and for the entire series. The children are divided into age groups. Figs. 3–6 show the concentrations of IgG, IgA, IgM and IgD in the individual children, and also the linear regression lines for boys and girls divided into two groups: A, healthy children with no allergic symptoms

Table 2. *IgG concentrations in mg/100 ml (arithmetic mean ± S.D., range and geometric mean). The values are given for group A (healthy children without allergic symptoms) and for the entire series*

| Age (years) | Healthy children without allergic symptoms | | | | All children | | | |
|---|---|---|---|---|---|---|---|---|
| | Boys | | Girls | | Boys | | Girls | |
| | Arithmetic mean ± S.D. (Range) | Geometric mean | Arithmetic mean ± S.D. (Range) | Geometric mean | Arithmetic mean ± S.D. (Range) | Geometric mean | Arithmetic mean ± S.D. (Range) | Geometric mean |
| 3 (2.5–3.5) | 896 ± 276 (6?1–1183) | 862 | 876 ± 143 (764–1050) | 866 | 821 ± 307 (621–1183) | 773 | 84? ± 157 (641–1050) | 830 |
| 4 (3.5–4.5) | 853 ± 112 (708–1000) | 847 | 1033 ± 192 (724–1331) | 1017 | 926 ± 229 (403–1430) | 902 | 1064 ± 231 (724–1562) | 1041 |
| 5–6 (4.5–6.5) | 900 ± 311 (533–1525) | 913 | 1031 ± 241 (70?–1303) | 1005 | 994 ± 286 (533–15?5) | 953 | 1107 ± 273 (70?–1558) | 1075 |
| 7–8 (6.5–8.5) | 1006 ± 221 (552–1289) | 980 | 1068 ± 163 (804–1225) | 1057 | 1008 ± 201 (552–1289) | 987 | 1076 ± 162 (771–1225) | 1064 |
| 9–10 (8.5–10.5) | 972 ± 134 (836–1258) | 965 | 1054 ± 90 (971–1204) | 1051 | 1076 ± 269 (843–1?58) | 1049 | 1077 ± 137 (945–1321) | 1070 |
| 11–12 (10.5–12.5) | 1169 ± ?46 (729–1674) | 1144 | 1335 ± 75 (1226–1396) | 1334 | 1228 ± 259 (729–1796) | 1?02 | 1304 ± 102 (1173–1419) | 1300 |
| 13–15 (12.5–15.5) | 1470 ± 346 (10?0–2430) | 1430 | 1562 ± 409 (1010–?653) | 1517 | 1494 ± 394 (949–2437) | 1448 | 1589 ± 39? (925–2653) | 1542 |

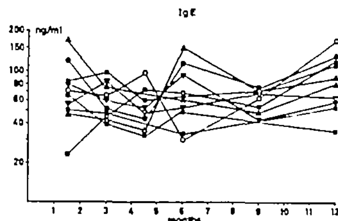



*Fig 1* IgE levels in 10 healthy children with no allergic symptoms from the age of 6 weeks to 1 year

of Mancini *et al* (12) but with some modifications (10) The IgE concentrations were estimated by the radio immunosorbent technique of Wide & Porath (13) as applied for IgE determination by Johansson *et al* (7) By this method IgE concentrations as low as 5 ng/ml could be measured The error of the method calculated as the standard deviation (S D) for duplicate analysis of the samples was about 15

As mentioned earlier (5) the concentrations of IgG IgA and IgM are not distributed in a Gaussian manner This is also true for IgD and IgE For this reason the statistical calculations were performed both on arithmetic values and on values transformed logarithmically to base 10 IgD amounts less than 1 mg/100 ml were calculated as 0 5 mg/100 ml The confidence limits given in tables and figures (95 interval) are calculated via the log values of the determinations For the statistical calculations the ages of the children at the time of sampling were estimated with an exactitude of hundredths of years The statistical analysis of the results was performed with the aid of a computor

## RESULTS

Fig 1 shows the development of IgE levels in 10 children between the age of 6 weeks and 1 year As can be seen in the figure the IgE levels rose slowly and without pronounced variations between different sampling occasions in the individual child At the age of 3 months these children showed a mean IgE value of 60 ng/ml serum at 6 months 72 ng/ml and at 1 year 91 ng/ml These values are in good agreement with previously reported findings (8)

In the six children for whom IgE determinations were made on pooled samples a mean IgE value of 33 ng/ml serum was found at the age of 2–4 days and 34 ng/ml at 2–3 weeks There appeared thus to be no rapid development of IgE levels during the first weeks of life

Fig 2 shows the individual IgE values in the 138 children of 2–15 years for whom a primary case history with regard to allergic manifestations was completely negative The frequency distribution of IgE was positively skewed but when the logarithmic values were graphed a fairly normal distribution was ob-

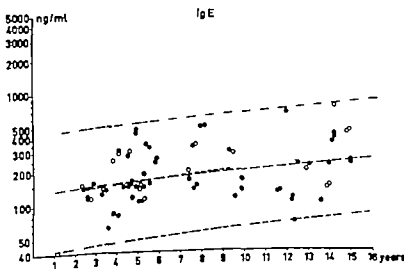



*Fig 2* IgE concentrations in 138 healthy children with no allergic symptoms Each child is represented by a symbol (● = boys ○ = girls) Regression lines (log IgE on age) for boys (——) and girls (– – – –) are superimposed as well as the confidence limits ( – ) for all the children

*Table 1. IgE concentrations in ng/ml (arithmetic mean ± S.D., range, geometric mean and confidence limits) in 132 healthy children divided into age groups*

| Age (years) | Healthy children without allergic symptoms | |
|---|---|---|
| | Arithmetic mean ± S.D. (Range) | Geometric mean (95 per cent confidence interval) |
| 2–2.5 | 137 ± 47 (73–185) | 129 (58–286) |
| 3 (2.5–3.5) | 140 ± 58 (64–260) | 129 (57–294) |
| 4 (3.5–4.5) | 178 ± 93 (67–308) | 154 (48–494) |
| 5–6 (4.5–6.5) | 09 ± 119 (63–487) | 183 (64–523) |
| 7–8 (6.5–8.5) | 251 ± 167 (65–535) | 199 (46–861) |
| 9–10 (8.5–10.5) | 256 ± 158 (69–530) | 209 (53–828) |
| 11–12 (10.5–12.5) | 239 ± 169 (68–715) | 196 (55–703) |
| 13–15 (12.5–15.5) | 330 ± 212 (54–840) | 268 (69–1040) |

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In Tables 2–5 the concentrations of the other four immunoglobulins G, A, M and D are given both for 132 children with no allergic manifestations or raised IgE values and for the entire series. The children are divided into age groups. Figs. 3–6 show the concentrations of IgG, IgA, IgM and IgD in the individual children and also the linear regression lines for boys and girls divided into two groups: A healthy children with no allergic symptoms

*Table 2. IgG concentrations in mg/100 ml (arithmetic mean ± S.D., range and geometric mean). The values are given for group A (healthy children without allergic symptoms) and for the entire series*

| Age (years) | Healthy children without allergic symptoms | | | | All children | | | |
|---|---|---|---|---|---|---|---|---|
| | Boys | | Girls | | Boys | | Girls | |
| | Arithmetic mean ± S.D. (Range) | Geometric mean | Arithmetic mean ± S.D. (Range) | Geometric mean | Arithmetic mean ± S.D. (Range) | Geometric mean | Arithmetic mean ± S.D. (Range) | Geometric mean |
| 3 (2.5–3.5) | 896 ± 276 (671–1183) | 867 | 876 ± 143 (764–1050) | 866 | 821 ± 307 (621–1183) | 773 | 842 ± 157 (641–1090) | 830 |
| 4 (3.5–4.5) | 853 ± 112 (708–1000) | 847 | 1033 ± 192 (724–1351) | 1017 | 926 ± 2.9 (603–1430) | 902 | 1064 ± 231 (724–1562) | 1041 |
| 5–6 (4.5–6.5) | 960 ± 311 (533–1525) | 913 | 1031 ± 41 (702–1303) | 1006 | 994 ± 286 (533–1525) | 953 | 1107 ± 273 (702–1558) | 1075 |
| 7–8 (6.5–8.5) | 1006 ± 221 (552–1289) | 980 | 1068 ± 163 (804–1225) | 1057 | 1008 ± 201 (552–1289) | 987 | 1076 ± 142 (771–1225) | 1064 |
| 9–10 (8.5–10.5) | 977 ± 134 (858–1258) | 965 | 1054 ± 90 (971–1704) | 1051 | 1076 ± 269 (842–1758) | 1049 | 1077 ± 137 (945–1321) | 1070 |
| 11–12 (10.5–12.5) | 1169 ± 346 (779–1674) | 1144 | 1335 ± 75 (1 6–1396) | 1334 | 1228 ± 259 (729–1756) | 1702 | 1304 ± 102 (1173–1419) | 1300 |
| 13–15 (12.5–15.5) | 1470 ± 306 (1030–2430) | 1430 | 156 ± 409 (1010–2653) | 1517 | 1484 ± 394 (949–2437) | 1448 | 1589 ± 397 (925–2653) | 1542 |

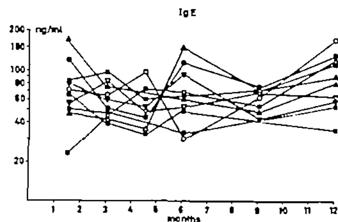



*Fig 1* IgE levels in 10 healthy children with no allergic symptoms from the age of 6 weeks to 1 year

of Mancini *et al* (12) but with some modifications (10) The IgE concentrations were estimated by the radio immunosorbent technique of Wide & Porath (13) as applied for IgE determination by Johansson *et al* (7) By this method IgE concentrations as low as 5 ng/ml could be measured The error of the method calculated as the standard deviation (S D ) for duplicate analysis of the samples was about 15

As mentioned earlier (5) the concentrations of IgG IgA and IgM are not distributed in a Gaussian manner This is also true for IgD and IgE For this reason the statistical calculations were performed both on arithmetic values and on values transformed logarithmically to base 10 IgD amounts less than 1 mg/100 ml were calculated as 0 5 mg/100 ml The confidence limits given in tables and figures (95 interval) are calculated via the log values of the determinations For the statistical calculations the ages of the children at the time of sampling were estimated with an exactitude of hundredths of years The statistical analysis of the results was performed with the aid of a computor

## RESULTS

Fig 1 shows the development of IgE levels in 10 children between the age of 6 weeks and 1 year As can be seen in the figure the IgE levels rose slowly and without pronounced variations between different sampling occasions in the individual child At the age of 3 months these children showed a mean IgE value of 60 ng/ml serum at 6 months 72 ng/ml and at 1 year 91 ng/ml These values are in good agreement with previously reported findings (8)

In the six children for whom IgE determinations were made on pooled samples a mean IgE value of 33 ng/ml serum was found at the age of 2–4 days, and 34 ng/ml at 2–3 weeks There appeared thus to be no rapid development of IgE levels during the first weeks of life

Fig 2 shows the individual IgE values in the 138 children of 2–15 years for whom a primary case history with regard to allergic manifestations was completely negative The frequency distribution of IgE was positively skewed but when the logarithmic values were graphed a fairly normal distribution was ob

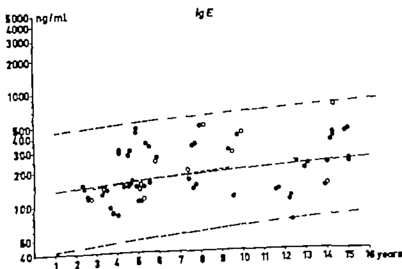



*Fig 2* IgE concentrations in 138 healthy children with no allergic symptoms Each child is represented by a symbol (• – boys o – girls) Regression lines (log IgE on age) for boys (——) and girls (- - - -) are superimposed as well as the confidence limits ( – ) for all the children

Table 5 *IgD concentrations in mg/100 ml (arithmetic mean ± S D range and geometric mean). The values are given for group A (healthy children without allergic symptoms) and for the entire series*

| Age years | Healthy children without allergic symptoms | | All children | | |
|---|---|---|---|---|---|
| | Arithmetic mean ± S D (Range) | Geometric mean | Arithmetic mean ± S D (Range) | Geometric mean | Number of children lacking IgD (<1 mg/100 ml) |
| 3 (2.5-3.5) | 0.95 ± 0.63 (<1-1.93) | 0.79 | 0.88 ± 0.60 (<1-1.93) | 0.73 | 9/13 = 69 |
| 4 (3.5-4.5) | 1.14 ± 0.83 (<1-3.17) | 0.91 | 1.33 ± 0.92 (<1-3.40) | 1.07 | 22/57 = 39 |
| 5-6 (4.5-6.5) | 1.11 ± 0.67 (<1-2.35) | 0.92 | 1.45 ± 1.16 (<1-5.94) | 1.12 | |
| 7-8 (6.5-8.5) | 2.14 ± 1.47 (<1-6.56) | 1.71 | 2.55 ± 1.76 (<1-8.34) | 2.07 | 9/76 = 12 |
| 9-10 (8.5-10.5) | 2.78 ± 2.42 (<1-9.11) | 1.95 | 2.52 ± 1.98 (<1-9.11) | 1.90 | |
| 11-12 (10.5-12.5) | 2.96 ± 1.65 (<1-6.41) | 2.68 | 3.22 ± 1.71 (<1-7.03) | 2.74 | |
| 13-15 (12.5-15.5) | 3.62 ± 3.71 (<1-20.15) | 2.45 | 3.92 ± 5.25 (<1-28.02) | 2.34 | 9/57 = 16 |

The IgG levels rose successively throughout childhood. At about 11–12 years the previously reported adult level (10) was attained on the whole and during puberty values even higher than adult values were reached on the average. This difference showed statistical significance ($p<0.01$) on comparing 59 children of 13–16 years with 64 adults from whom samples were also taken for the study of poliomyelitis immunity. The girls showed higher mean IgG concentrations throughout than the boys. Regression analyses with regard to age and sex showed a significant relationship between age and IgG concentration ($p<0.001$). The sex difference was statistically significant both in the group of healthy children with no allergic symptoms ($p<0.025$) and in the series as a whole ($p<0.005$).

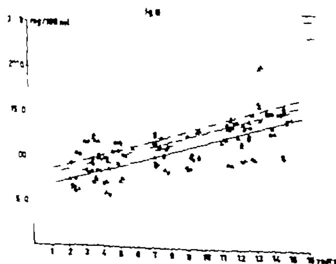


Fig. 3. IgG concentrations in all children of the series. Each child is represented by a symbol ( = "healthy" boys, = "healthy" girls, ▲ = "other" boys, △ = "other" girls). Linear regression lines (IgG on age and sex) are superimposed.

Table 3 *IgA concentrations in mg/100 ml (arithmetic mean, S D range and geometric mean) The values are given for group A (healthy children without allergic symptoms) and for the entire series*

| Age (years) | Healthy children without allergic symptoms: Arithmetic mean ± S D (Range) | Geometric mean | All children: Arithmetic mean ± S D (Range) | Geo-metric mean |
|---|---|---|---|---|
| 3 (2 5–3 5) | 62 6±32 7 (21 8–140 4) | 55 3 | 58 2±32 0 (21 8–140 4) | 50 8 |
| 4 (3 5–4 5) | 73 0±22 8 (38 8–101 0) | 69 3 | 81 7±30 9 (29 6–144 6) | 75 8 |
| 5–6 (4 5–6 5) | 101 0±61 5 (30 6–274 3) | 86 4 | 94 0±52 3 (30 6–274 3) | 83 3 |
| 7–8 (6 5–8 5) | 95 2±31 0 (55 8–162 0) | 90 6 | 100 7±30 7 (55 8–164 8) | 96 1 |
| 9–10 (8 5–10 5) | 102 5±31 1 (47 2–143 0) | 97 4 | 108 8±30 4 (47 2–148 8) | 103 8 |
| 11–12 (10 5–12 5) | 113 1±49 2 (41 8–274 9) | 104 9 | 125 5±51 2 (41 8–274 9) | 116 5 |
| 13–15 (12 5–15 5) | 132 7±86 6 (60 5–465 7) | 115 2 | 130 2±72 9 (55 9–465 7) | 116 5 |

( healthy ) and B other children included in the series ( others ) In Figs 7–10 the curved regression lines (obtained by regression analyses on logarithmic values) are compared with the linear regression lines and the 95 per cent confidence intervals for the respective immunoglobulins are given

The numericɑl results of the regıession analyses mentioned above are given in Tables 6 and 7

Table 4 *IgM concentrations in mg/100 ml (arithmetic mean S D range and geometric mean) The values are given for group A (healthy children without allergic symptoms) and for the entire series*

| Age (years) | Healthy children without allergic symptoms: Boys: Arithmetic mean ± S D (Range) | Geo metric mean | Girls: Arithmetic mean ± S D (Range) | Geo metric mean | All children: Boys: Arithmetic mean ± S D (Range) | Geo-metric mean | Girls: Arithmetic mean ± S D (Range) | Geo-metric mean |
|---|---|---|---|---|---|---|---|---|
| 3 (2 5–3 5) | 72 9±8 8 (61 3–81 8) | 72 5 | 92 6±43 0 (41 0–163 3) | 84 4 | 67 4±15 6 (40 0–81 8) | 65 6 | 89 8±39 9 (41 0–163 3) | 82 7 |
| 4 (3 5–4 5) | 66 2±33 1 (29 3–132 0) | 60 0 | 120 2±66 5 (39 0–241 3) | 104 9 | 67 6+26 4 (29 3–132 0) | 63 3 | 115 1±55 2 (39 0–241 3) | 104 1 |
| 5–6 (4 5–6 5) | 76 2±33 6 (43 0–174 8) | 71 3 | 90 9±18 6 (65 0–111 3) | 89 2 | 73 1+31 0 (36 5–174 8) | 68 [illegible] | 90 9±16 2 (65 0–111 3) | 89 6 |
| 7–8 (6 5–8 5) | 73 2±24 6 (32 3–111 5) | 69 1 | 86 1±23 5 (59 5–117 5) | 83 4 | 68 5±22 9 (32 3–111 5) | 64 9 | 80 7±23 2 (37 0–117 5) | 77 3 |
| 9–10 (8 5–10 5) | 78 0±24 8 (45 0–114 8) | 74 6 | 94 1+32 4 (68 0–134 3) | 89 9 | 77 3±34 5 (45 0–163 0) | 71 3 | 99 9±28 8 (40 5–134 3) | 96 2 |
| 11–12 (10 5–12 5) | 79 2±37 1 (79 8–189 5) | 72 6 | 108 1±42 9 (80 5–172 0) | 102 8 | 79 5±34 0 (79 8–189 5) | 73 9 | 94 3±36 6 (62 8–172 0) | 90 0 |
| 13–15 (12 5–15 5) | 83 9±30 2 (39 8–124 9) | 78 3 | 103 1±39 5 (54 5–208 8) | 95 4 | 77 1±30 3 (26 7–124 9) | 71 0 | 99 8±36 9 (54 5–208 8) | 93 8 |

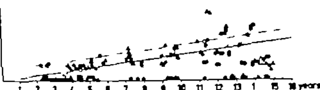



*Fig 6* IgD concentrations in all children of the series Each child is represented by a symbol ( = healthy" boys o = healthy" girls ▲ = other" boys △ = other' girls) Linear regression lines (IgD on age and sex) are superimposed

Regression analyses showed a statistically significant relationship between age and IgD level ($p < 0\,001$) but no sex difference

## DISCUSSION

The subjects called for the investigation were selected randomly in such a way that the series as a whole should be representative of the population in a medium sized Swedish town Since as many as 84 per cent of the children attended for the investigation the paediatric series reported here can be regarded as essentially unselected This is a great advantage in a study of the immunoglobulin development during childhood considerable large difficulties are often encountered in collecting a representative series especially of children after preschool age and adolescents

In general the regression analyses performed on logarithmic immunoglobulin values gave a better fit of regression than the regression analyses performed on arithmetic values (Tables 6 and 7) This seems to be particularly true for IgA and IgD For IgE the two kinds of regression analysis gave about the same

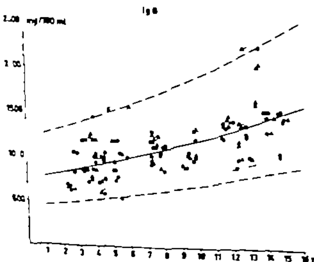



*Fig 7* IgG concentrations in all children of the series In the figure the curved regression line obtained by regression analyses on logarithmic values (———) is compared with the linear regression line ( ) The regression lines satisfy the whole series The 95 per cent intervals are given for boys (— —) and girls ( - )

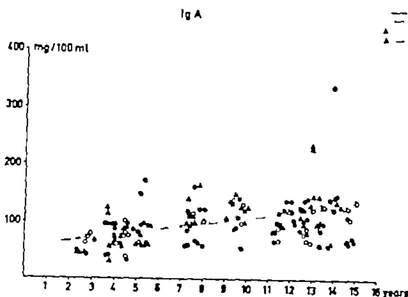



*Fig 4* IgA concentrations in all children of the series Each child is represented by a symbol ( = healthy boys o = healthy girls ▲ = other boys △ = other girls) Linear regression lines (IgA on age and sex) are superimposed

There seemed to be no systematic sex difference with regard to IgA The IgA levels rose gradually throughout childhood and in the age group 13–15 years they had still not attained the adult level Regression analyses showed a significant relationship between age and IgA level ($p<0.001$) but no significant sex difference

With respect to IgM sex differences were found throughout the IgM level in the girls was 118–173 per cent of that in the boys and the greatest difference was found at the age of 4 years The weighted mean value for boys and girls reached the previously reported value for adults (10) as early as at 4 years Regression analyses indicated no significant importance of age for the IgM concentrations in the age groups concerned (2–15 years) On the other hand a significant sex difference was found This was true for group A ( healthy ) as well as for group B ( others ) and for the series as a whole ($p<0.001$)

IgD was lacking (<1 mg/100 ml) in 39 per cent of the children between 4 and 6 years of age a figure which agrees well with previous findings (6) From the age of 7–8 years and onwards IgD was lacking in 12–16 per cent of the children

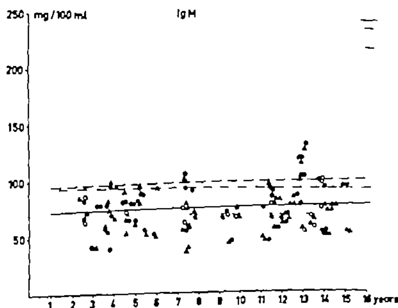



*Fig 5* IgM concentrations in all children of the series Each child is represented by a symbol ( = healthy boys o = healthy girls ▲ = other boys △ = other girls) Linear regression lines (IgM on age and sex) are superimposed

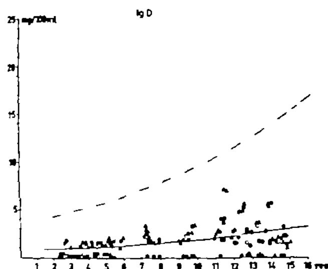



*Fig. 10.* IgD concentrations in all children of the series. In the figure the curved regression line obtained by regression analysis on logarithmic values (———) is compared with the linear regression line ( ). The regression lines satisfy the whole series. The 95 per cent interval (-·—·-) is given for the whole series.

Table 6. *Results of regression analyses with regard to age for the respective immunoglobulins. The statistical calculations were performed both on arithmetic values and on logarithmic values. t = age in years. Significance of regression coefficients is denoted by asterisks*

| Regression on age | | S | R |
|---|---|---|---|
| *Arithmetic values* | | | |
| All children | IgG = 700 4 + 54 8 t | 300 4 | 0 36 |
| | IgA = 58 6 + 5 37 t | 51 4 | 0 15 |
| | IgM = 80 2 + 0 46 t | 35 1 | 0 004 |
| | IgD = 0 192 0 ∠67 t | 3 00 | 0 12 |
| Group A ( healthy ) | IgE 120 8 + 14 1 t | 156 6 | 0 13 |
| All boys | IgG 676 7 + 52 1 t* | 296 6 | 0 34 |
| | IgA 63 8 4 94 t | 46 2 | 0 16 |
| | IgM = 67 8 + 0 77 t | 29 5 | 0 02 |
| | IgD 0 338 0 239 t | 2 55 | 0 13 |
| All girls | IgG = 736 4 58 t | 295 6 | 0 41 |
| | IgA 51 7 + 5 93 t | 58 4 | 0 15 |
| | IgM 97 5 0 04 t | 37 9 | 0 00002 |
| | IgD 0 011 0 303 t | 3 57 | 0 11 |
| *Healthy boys | IgE 136 0 11 7 t | 144 1 | 0 10 |
| Healthy girls | IgE = 100 7 17 4 t | 174 7 | 0 16 |
| *Logarithmic values* | | | |
| All children | log IgG = 2 87566 0 0?01 t | 0 10595 | 0 38 |
| | log IgA 1 76766 0 0?35 t | 0 18646 | 0 21 |
| | log IgM = 1 86974 0 0025 t | 0 17102 | 0 0035 |
| | log IgD 0 86133 1 0 0415 t | 0 34615 | 0 19 |
| Group A ( healthy ) | log IgE = 2 10016 0 0223 t | 0 ?6663 | 0 11 |
| All boys | log IgG = 2 85663 + 0 0.0? t | 0 10996 | 0 36 |
| | log IgM = 1 81096 0 0035 t | 0 16364 | 0 0073 |
| All girls | log IgG = 90051 0 0199 t | 0 09547 | 0 44 |
| | log IgM = 1 95199 + 0 0011 t | 0 15725 | 0 0009 |
| *Healthy boys | log IgE = 2.11397 + 0 0.00 t | 0 ?7083 | 0 09 |
| Healthy girls | log IgE = 2 08 07 + 0 0 53 t | 0 26479 | 0 13 |

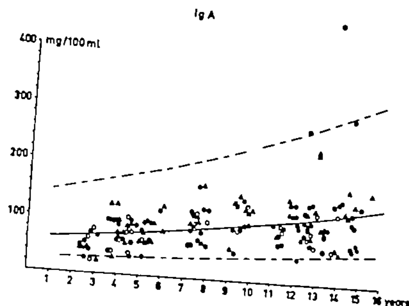



*Fig 8* IgA concentrations in all children of the series In the figure the curved regression line obtained by regression analysis on logarithmic values (———) is compared with the linear regression line ( ) The regression lines satisfy the whole series The 95 per cent interval (– — –) is given for the whole series

goodness of fit of regression The 95 per cent confidence intervals given in Figs 2 and 7–10 are calculated via the regression analyses on log values and seem to be fairly well adapted for practical use

One of the main purposes of the present investigation was to study the IgE development throughout childhood in children who had had no history of allergic manifestations in order to establish mean values and normal variations for different age groups The previously performed investigation of IgE levels in children (8) comprised a relatively small number of subjects up to the age of 5 years It is found from the present results that some of the conclusions drawn from that study need to be reconsidered Thus there appears to be no especially rapid IgE development during early infancy The IgE values rise slowly and evenly throughout childhood and have still not reached the maximal level during the years of puberty

During early adulthood the mean IgE levels seem to increase further to some extent and subsequently become stable or decrease somewhat (8) Thus the IgE development seems to be essentially similar to that of IgA

Of the 138 children with no known allergic manifestations six showed IgE concentrations which deviated clearly from those of the other

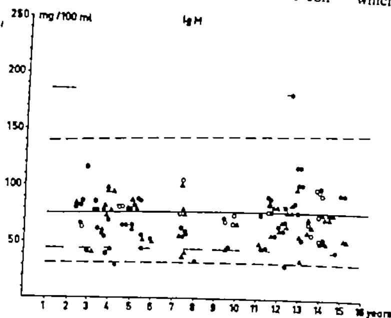



*Fig 9* IgM concentrations in all children of the series In the figure the regression line obtained by regression analysis on logarithmic values (———) is compared with the linear regression line ( ) The regression lines satisfy the whole series The 95 per cent intervals are given for boys (——) and girls (– – –)

was statistically significant ($p<0.001$) Twenty two of the 32 children had IgE values above the average but compared with the normal distribution in Fig 2 only 10 children had clearly raised values These results will be discussed in greater detail in a future publication (3)

Whereas the IgE results in the tables and figures refer only to those children who were free from allergic symptoms and were also healthy in other respects (group A) the results for the other immunoglobulins are also given for the other children in the series (group B) and for the series in its entirety Group B ("others") is of a heterogeneous composition but consists mainly of children who had had more or less definite allergic manifestations but otherwise had been essentially healthy

In a comparison between group A and group B somewhat higher mean concentrations of IgG and IgD were found in the latter group but these differences were not statistically significant When however the healthy children were compared with those who were considered to have atopic conditions significantly higher IgG values ($p<0.05$) and IgD values ($p<0.025$) were found in the latter That persons with atopic diseases have on the average significantly higher IgD values than those without such conditions was shown by Kohler & Farr (11) These authors considered that the higher IgD concentrations in the former were probably not a result of the presence of reaginic antibodies but had another cause They also found higher mean IgG concentrations in persons with atopic conditions although this difference was not statistically significant

One possible explanation for the higher IgG and IgD levels in the children with atopic conditions is a higher frequency of infections In the present series these children showed no higher concentrations of IgA and IgM however than the healthy children A previous investigation of infants during their first year of life (2) showed that recurrent infections of different kinds affected predominantly the levels of IgM and IgA Hitherto no specific antibody activity within the IgD class has been reported and no definite explanation for the higher IgD levels in the children with allergic manifestations can be given

It is of interest to note that the girls had higher IgG concentrations throughout than the boys This sex difference was not large but it was statistically significant both within the group of healthy children and in the series as a whole As far as we know previously published investigations have shown no systematic sex difference with regard to IgG (1 4)

The girls had also higher IgM levels throughout than the boys and for some reason this difference was most pronounced at the age of 4 years Regression analysis showed a statistically highly significant sex difference for this immunoglobulin A systematic sex difference has been demonstrated previously in adults (4 10) and also in children from the ages of about 7–8 years (1 4) There seem to be no sex differences during infancy as far as has been found hitherto (1 2)

The finding of higher IgG concentrations in adolescents than in adults has also been reported by Allansmith *et al* (1)

On comparing the development of IgG IgA and IgD in the two groups of children ("healthy" and "others") practically parallel regression lines are found (Figs 3–4 and 6) This finding indicates that the age factor is of great and obviously very constant importance for the development of these immunoglobulins

The lowest frequency of children lacking IgD was found in the age groups before puberty It is of interest to compare this finding with that of Johansson *et al* (10) They found that in adults IgD was not detectable in 12 per cent of persons of ages 20–35 years while older persons lacked IgD in a higher frequency (23–25 per cent)

## SUMMARY

In a series comprising 215 children of ages 2–15 years representative of the population in a medium sized Swedish town determinations

Table 7 *Results of regression analyses with regard to age and sex for the respective immunoglobulins The statistical calculations were performed both on arithmetic values and on logarithmic values t = age in years X = 1 for boys X = 2 for girls Significance of regression coefficients is denoted by asterisks*

| Regression on age and sex | | S | $R^2$ |
|---|---|---|---|
| *Arithmetic values* | | | |
| All children | IgG = 541 4 + 54 6 t*** + 113 6 X** | 295 9 | 0 38 |
| | IgA = 63 3 + 5 36 t*** − 3 30 X | 51 5 | 0 16 |
| | IgM = 47 3 + 0 44 t + 23 26 X*** | 33 2 | 0 11 |
| | IgD = −0 159 + 0 266 t + 0 250 X | 3 00 | 0 12 |
| Group A ( healthy ) | IgG = 514 0 + 53 4 t + 119 1 X | 282 4 | 0 40 |
| | IgA = 60 8 + 5 31 t*** − 2 53 X | 57 5 | 0 13 |
| | IgM = 49 4 + 0 65 t + 22 36 X*** | 34 3 | 0 10 |
| | IgD = 0 089 + 0 264 t − 0 595 X | 2 22 | 0 20 |
| | IgE = 103 3 + 14 03 t*** + 12 74 X | 157 1 | 0 13 |
| Group B ( others ) | IgG = 590 7 + 55 6 t*** + 106 0 X | 315 5 | 0 36 |
| | IgA = 67 8 + 5 39 t* * − 4 48 X | 41 1 | 0 23 |
| | IgM = 43 8 + 0 19 t + 24 5 X* * | 31 5 | 0 13 |
| | IgD = −0 511 + 0 264 t* + 0 749 X | 3 97 | 0 08 |
| *Logarithmic values* | | | |
| All children | log IgG = 2 81367 + 0 0201 t + 0 0438 X * | 0 10395 | 0 40 |
| | log IgA = 1 80083 + 0 0235 t* − 0 0235 X | 0 18656 | 0 21 |
| | log IgM = 1 69964 + 0 0025 t + 0 1203 X | 0 16070 | 0 12 |
| | log IgD = 0 84705 − 1 + 0 0415 t*** + 0 0101 X | 0 34696 | 0 20 |
| Group A ( healthy ) | log IgG = 2 80277 + 0 0197 t * + 0 0466 X** | 0 09835 | 0 43 |
| | log IgA = 1 80341 + 0 0225 t − 0 0288 X | 0 19657 | 0 19 |
| | log IgM = 1 71982 + 0 0034 t + 0 1091 X*** | 0 16285 | 0 11 |
| | log IgD = 0 82563 − 1 + 0 0475 t − 0 0323 X | 0 33398 | 0 26 |
| | log IgE = 2 08139 + 0 0222 t** + 0 0136 X | 0 26758 | 0 11 |
| Group B ( others ) | log IgG = 2 83303 + 0 0203 t*** + 0 0397 X | 0 11225 | 0 37 |
| | log IgA = 1 79868 + 0 0247 t*** − 0 0144 X | 0 17059 | 0 76 |
| | log IgM = 1 66548 + 0 0012 t + 0 1376 X * | 0 15748 | 0 16 |
| | log IgD = 0 89501 1 + 0 0308 t** + 0 0776 X | 0 36188 | 0 12 |

subjects In these children a further case history was taken whereupon the following was found one boy 7 years old had not had any typical allergic symptoms but felt unwell when eating shrimps or lobster This boy had a brother with flexural eczema One girl 12 years old, had had repeated attacks of pseudo croup up to the age of 7 years a brother of hers had had slight symptoms of asthma One boy 11 years old had never had any allergic manifestations but his mother suffered from eczema One girl 4 years old had never had any allergic symptoms and had no known atopic heredity A brother of hers had been treated for ascariasis but the girl herself had had no such symptoms In the remaining 2 children the further case history provided no positive evidence of either allergic symptoms atopic heredity or ascariasis

It is known that a high percentage of Ethiopian children have greatly increased IgE concentrations which is probably explainable by infestations with different kinds of parasites (9) Children with proved infestations of Ascaris lumbricoides thus had 28 times higher IgE concentrations than healthy Swedish children of the same age while children without proved ascariasis had considerably lower but still distinctly raised IgE values (about 5 times) It seems possible that isolated raised IgE values in a series of subjects with a negative case history might be explained by ascariasis or perhaps other infestations

In the 32 children whose case history indicated an atopic constitution high mean IgE levels were found as expected these were more than 3 times the average in healthy children of corresponding ages This difference

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SHORT COMMUNICATION

# SEVERE FAMILIAL LACTOSE INTOLERANCE—A GASTROGEN DISORDER?

N O BERG A DAHLQVIST T LINDBERG and W v STUDNITZ

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Congenital lactose intolerance in infancy is usually divided into two forms hereditary *lactase deficiency* (alactasia or hypolactasia) with osmotic diarrhoea on lactose ingestion and *severe familial lactose intolerance* which is a more serious disease characterized by vomiting failure to thrive dehydration disaccharidaria and amino aciduria (for ref see Holzel 1967 (4)) It has been suggested that the lactose intolerance in the latter disease is due to a temporary lactase deficiency since normal lactase activity has been found in the intestinal mucosa after recovery (5) However direct proof supporting this suggestion is as far as we know still lacking

In this preliminary report some observations made on a boy suffering from symptoms indicating lactose intolerance of the severe familial form will be presented

The boy (T K 68 02 09) first child of healthy parents and born at term was referred to the Department of Pediatrics Malmo General Hospital at 10 days of age because of vomiting and decrease in weight During the following weeks he presented main symptoms as excessive vomiting failure to thrive lactosuria and amino aciduria (Fig 1) An X ray of the stomach and small intestine at the age of two weeks revealed a very slight elongating and narrowing of the pyloric canal A second X ray of the stomach at the age of five weeks was quite normal The stools were normal and Clinitest of faeces negative and pH of faeces 6–7

Various investigations concerning the urinary excretion of carbohydrates and amino acids were performed

*Lactose* was calculated from the increase in free galactose (assayed with galactose dehydrogenase (3 9)) after incubation with a fungal lactase

*Sucrose* was calculated from the increase in free glucose (assayed with TRIS-glucose oxidase (1)) after incubation with yeast invertase Urine was filtered through anion exchange resin before analysis (10)

*α-amino nitrogen* in urine was measured as described by Khachadurian *et al* (6)

*Amino acids* were determined as described by one of us (11)

The results are summarized in Table 1 During breast milk feeding and after per oral lactose tolerance tests severe lactosuria was found When lactose was administered intraduodenally however practical no lactosuria occurred Administration of sucrose *per os* (Nutramigen or sucrose tolerance test) resulted in sucrosuria. All the tolerance tests resulted in an increased excretion of cystathionine This may be interpreted as an impaired liver func

were made of the 5 immunoglobulins IgG IgA IgM IgD and IgE The children were divided with regard to the occurrence of allergic symptoms and to their general state of health The results were subjected to extensive statistical calculations including regression analyses with regard to age and sex for the different immunoglobulins A longitudinal study of the IgE development during the first year of life was also carried out

Mean values and normal variations for IgE at different ages are presented The IgE development during childhood was found to be essentially similar to that of IgA

Adult IgG levels were reached, on the whole at the age of 11–12 years and a further IgG increase was noted in children during puberty The girls had higher IgG concentrations throughout than the boys this sex difference is not large but is statistically significant

The IgA levels rose gradually throughout childhood and had still not reached an adult level during puberty No sex difference was found for IgA

Adult IgM levels were attained by the age of 4 years The girls showed higher IgM concentrations throughout than the boys and this difference is statistically significant

A statistically significant relationship was found between age and IgD level IgD was lacking from the age of 7–8 years in 12–16 per cent of the children a somewhat lower figure than has been reported previously for adults No statistically significant sex difference was found Children with atopic conditions had higher IgD levels than completely healthy children this difference was statistically significant

## ACKNOWLEDGEMENTS

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The authors wish to express their thanks to Associate Professor Claes Hogman for his interest in this investigation and for the facilities of his department placed at our disposal We also thank Assistant Professor Hans Bennich for IgE preparations Associate Professor Gosta Öberg and Associate Professor Lars Wranne for their positive interest in and practical support of this investigation Mrs Ulla Berg RN for her help in the collection of samples Goran Enholm M A for his help with the statistical analyses, and Miss Birgitta Larsson Miss Lilian Poubloel Mrs Stina Soderlund and Miss Britt Ytterberg for skilful technical assistance

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*Key words* Immunoglobulin levels children atopic disorders

It is clear from these studies that in this infant, presenting the signs and symptoms of severe familial lactose intolerance the intestinal disaccharidase activities—including lactase and sucrase activity—were within normal ranges in the acute phase of the disease. This does not support the idea that the disease is due to a temporary lactase deficiency. Our observations suggest a defect localized to the gastric mucosa resulting in an abnormal resorption of lactose (and apparently also other disaccharides) in the stomach.

A full account of this case will be published.

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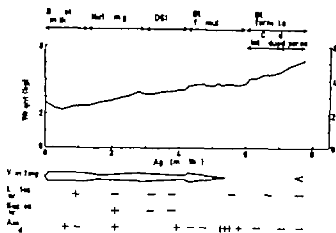


*Fig 1* Survey of clinical course DSI a monosaccharide formula manufactured by Nestle kindly supplied by Professor B Lindquist Glucose formula beside the sugar similar to fructose formula (8) Citrido citric acid milk Citrido intraduodenally tube feeding X ray control

tion in combination with an increased need for vitamin $B_6$ (11) As seen in Fig 1 the boy received citric acid milk *per os* at about the age of 8 months However after some weeks he began to vomit decreased in weight and got moderate lactosuria The diet was changed to glucose formula but no weight increase occurred When citric acid milk again was administered intraduodenally through a feeding tube for a few days a rapid increase in weight was noted Lactose was then not excreted in the urine

Small intestinal biopsies were performed at 6 and 20 weeks of age The specimens were studied with regard to morphology disaccharidase activity (2) and dipeptidase activity (7) The results are given in Table 2

Table 1 *Urinary excretion of disaccharides and amino acids*

| Age (months) | Diet | Tolerance test[a] | Blood glucose rise (mg/100 ml) | Lactose (mg/100 ml) | Sucrose (mg/100 ml) | Amino acids (mg α amino N/g creatinine) |
|---|---|---|---|---|---|---|
| 1 | Breast milk | — | | 525 | — | 214 |
| 2 | Nutramigen | — | | 8 | 810 | 720 |
| 2 | Nutramigen | Lactose per oral | >40 | 462 | — | 250 (cystathioninuria) |
| 2½ | Nutramigen | Sucrose per oral | >40 | | 734 | 569 (cystathioninuria) |
| 3½ | DSI[b] | — | | 1 2 | 0 | 340 |
| 3½ | DSI[b] | Lactose intraduodenal[c] | >40 | 28 4 | — | 357 (cystathioninuria) |
| 3¾ | DSI[b] | Lactose per oral | >40 | 400 | — | 284 (cystathioninuria) |

[a] Two g/kg bodyweight
[b] For explanation see Fig 1
Lactose was administered through a feeding tube in pars descendens of duodenum under flouroscopic control

Table 2 *Morphology disaccharidase and dipeptidase activities in small intestinal mucosa*

| Age (weeks) | Site | Morphology: Macroscopic | Morphology: Microscopic | Lactase activity (units/g protein) | Sucrase activity (units/g protein) | Maltase Isomaltase Trehalase activities | Dipeptidase activities |
|---|---|---|---|---|---|---|---|
| 6 | Flexura duodeno jejunalis | Leaves and ridges focus of flat mucosa | Small focal abnormality with plasma cells | 15 8 | 47 8 | Within normal limits | Within normal limits |
| 20 | Flexura duodeno jejunalis | Leaves | Normal | 24 8 (9–98)[a] | 29 3 (26–138)[a] | Within normal limits | — |

[a] Normal ranges

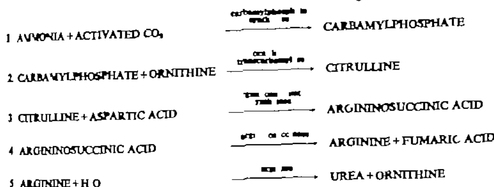



*Fig 1* Simplified urea cycle

(trace) 2 to 4 erythrocytes and 1 to 2 leukocytes h p f and some casts. With the phenistix reaction a brown discoloration was observed while the 2–4 dinitrophenylhydrazine reaction gave a yellowbrownish color. The Brand reaction was negative. True blood glucose was 125 mg per 100 ml serum calcium was 9.5 mg per 100 ml. In the cerebrospinal fluid the glucose and protein levels were normal; there were no cells. Bilateral subdural puncture was negative. The patient was immediately placed in an incubator and feeding consisted exclusively of intravenous fluids (1500 ml/ 4 hrs/m²). Metabolic acidosis could initially be corrected by Tris buffer. The clinical condition however quickly deteriorated and the apathy and stupor became extreme. From time to time myoclonic movements of the face, the tongue and the extremities were observed. A respiratory arrest on the fifth day necessitated intubation and artificial ventilation. Striking oliguria (7 ml only in the last 4 hours) and oedema appeared. Blood urea nitrogen was 18 mg per 100 ml creatinine 1.35 mg per 100 ml sodium 143 mEq per liter and potassium 8 mEq per liter. Urinary urea output on November 21 was 1.79 g/24 hrs. The patient died on the sixth day of life after a cardiac arrest (November 22 1966).

*Autopsy* (Prof Dr H Roels Department of Pathology Rijksuniversiteit Gent) revealed a diffuse bilateral pneumonia. Other findings which were interpreted as secondary phenomena were anoxemic subepicardial and subpleural bleeding, subarachnoidal bleeding in the left parietal area and depletion of thymocytes in the thymus. Macroscopically the brain was normal. Microscopic examination disclosed only a discrete congestion. No crystalline material was found in the different tissues.

*Family history*: both parents are healthy. There is no consanguinity. Their first child, a four-year-old girl, has developed normally from birth on. Their second child, a girl born at full term, developed normally until the fourth day of life when she was admitted to another hospital with respiratory distress and convulsions. She died on the fifth day. No biochemical data are available. The third child is the

## BIOCHEMICAL INVESTIGATIONS

### *Methods*

Aminoacids in blood and urine were studied by means of unidimensional paperchromatography according to the technique of Scriver *et al* (24). Brain aminoacids were estimated qualitatively by the method of Adriaenssens *et al* (1) and compared to those of supposedly normal brain tissue. A quantitative study of the aminoacids in urine, blood, cerebrospinal fluid, faeces, brain and renal tissue was performed by column chromatography (Technicon analyser) (10).

For the ASA assay in the urine, samples were acidified to pH 2, then boiled for 2 hours to convert free ASA to the more stable anhydrides (27). Since ethanolamine is eluted at the same position as the C anhydride, the calculated amount of ASA possibly exceeded the true value by approximately 5%. Urinary excretion of the α-ketoacids was estimated by thin layer chromatography using the method of Ronkainen (22) after extraction according to Kaser *et al* (14).

Argininosuccinase activity in blood cells was assessed by the method of Tomlinson & Westall (25).

The same technique was used for measurement of the activity of argininosuccinase and arginase in the kidney tissue. A 10% w/w homogenate was prepared in cold distilled water and was centrifuged at 3000 r p m for 15 minutes. The supernatant fraction was dialyzed for 2 hours at 4 C against distilled water and was centrifuged again. Linearity of the activity of the kidney enzymes as a function of time (up to 60 minutes) and of protein concentration (up to 4 mg per ml) was controlled. Three control human kidneys and a rat kidney were processed in parallel.

The argininosuccinase activity in gray brain matter was estimated by the method of Ratner (21) as modified by Tomlinson & Westall (25) except that the incubation time was extended to 6 hours (15).

All samples were stored at −30 C until required for analysis. It must be stated that the enzymatic activity in the renal tissue was assessed after a storage period at −30 C of 25 months. Storage during months at −30 C did not result in any gross loss

*Acta Pædiat Scand 58* 528–534 1969

CASE REPORT

# ARGININOSUCCINIC ACIDURIA

## *Neonatal Variant with Rapid Fatal Course*

D CARTON F DE SCHRIJVER J KINT J VAN DURME and C HOOFT

*From the Department of Pediatrics Rijksuniversiteit Gent Belgium*

Argininosuccinic aciduria is a rare inborn error of the urea cycle metabolism (Fig 1) Due to a deficient argininosuccinase activity the normal cleavage of argininosuccinic acid (ASA) into arginine and fumaric acid does not occur The deficiency has been demonstrated in blood cells (17 20 25) liver (17 19) and brain tissue (15) Consequently accumulation of ASA and in some cases, of its immediate precursor citrulline occurs in the blood, the cerebrospinal fluid and especially in the urine Ammonia levels may also be increased (17 20 23) The unexpected adequate production of urea in these patients has been explained by residual enzyme activity (19) by the existence of an alternative pathway or by isoenzyme activity The latter hypothesis is supported by the finding in one patient of a normal argininosuccinase activity in the renal tissue (8) The clinical symptoms which generally have their onset or become prominent in late infancy or childhood are mental retardation convulsions, sometimes hepatomegaly periods of coma and ataxia, brittle hairs and nails They are ascribed to the elevated ASA levels as well as to a possible arginine deficiency and chronic ammonia intoxication The recommended therapy consists of frequent small low protein meals in association with citric acid or another acid (17, 28)

This paper deals with a case of neonatal argininosuccinic aciduria differing from the classical form by its fulminant course with very early death An older sibling of the proband died of a similar disorder at the age of five days but no diagnosis was established at that time

Our view that the neonatal form of argininosuccinic aciduria represents a separate entity was strengthened by the publication by Baumgartner *et al* (4) of a similar case, which appeared when this paper was in preparation

## CASE REPORT

A girl aged four days was urgently admitted on November 20 1966 to the paediatric department because of poor sucking groaning and increasing respiratory distress in the last few hours Pregnancy and delivery had been uneventful Birth weight was 3300 g The baby cried immediately and no cyanosis was observed Breast feeding was started on the second day of life without any difficulty Behaviour had been normal until the fourth day of life when normal reactions progressively decreased and groaning with slight signs of respiratory distress appeared

On admission the infant groaned continuously discrete intercostal retractions and flaring of the alae nasi were present The most striking feature however was the pronounced apathy and hypotonia The child remained motionless without reaction to exogenous stimuli The Moro sucking and grasping reflexes were absent There was no bulging of the fontanel The head circumference was 32 8 cm The auscultation of heart and lungs was normal The spleen and liver were not enlarged The urine which diffused no peculiar smell contained small amounts of protein (approximately 1 g per 1000 ml) glucose

Table 3 *The argininosuccinase activity in blood cells from the patient family members and normal controls*

(expressed as μ mol urea produced per hour per g haemoglobin)

| | |
|---|---|
| Patient | 0 |
| Father | 1 4 |
| Mother | 1 3 |
| Sibling | 1 6 |
| Own control child | 4 2 |
| Normal subjects (*n* 25) | 1 9–6 9 |

*Ketoacids* The α ketoaciduria estimated by thin layer chromatography was normal The α ketoglutaric acid excretion was 8 5 mg per 24 hours and the pyruvic acid excretion 0 85 mg per 24 hrs No abnormal ketoacids were present

*Ammonia* Accurate quantitative data were not available On column chromatography however it appeared clearly that in comparison to a normal control child of the same age high to very high ammonia levels were present in the blood and liquor Although no comparative data on ammonia levels in human tissues are available the ammonia content of brain tissue and renal tissue probably also was increased

*Enzymatic study* No argininosuccinase activity was demonstrable in blood cells of the patient In both parents and their healthy daughter the enzymatic activity was intermediary between that of the propositus and that of a normal control Our data are comparable to those found by other authors in patients heterozygotes and normal controls (Table 3)

Table 4 *The argininosuccinase and arginase activity in kidney tissue from the patient and control children*

(expressed as mmol of urea formed per hour per mg protein)

| Subject | Age at death | Argininosuccinase activity | Arginase activity |
|---|---|---|---|
| Control 1 | days | 77 5 | 1130 |
| Control 2 | 5 months | 114 1 | 900 |
| Control 3 | 12 days | 71 7 | 950 |
| Patient | 6 days | 0 5 | 1440 |

In the gray brain matter the argininosuccinase activity was estimated at about 2 7 of that found in the brain of normal controls (15) No argininosuccinase activity could be demonstrated in the patient's kidney tissue The arginase activity fell within the normal range or even exceeded slightly the mean controls (Table 4)

## DISCUSSION

An inborn error of metabolism was suspected in a four-days old child who progressively developed respiratory distress and cerebral involvement and who died at the age of six days The positive family history reinforced our suspicion a sibling of the proband died earlier of a similar disorder

Metabolic disorders with possible clinical symptoms in the first days of life and frequently with rapid fatal course are the maple syrup urine disease the hyperglycinaemia type I and II the isovaleric acidemia and to a lesser degree the galactosaemia

The clinical symptoms as presented by the patient could to a certain extent have been those of the classical form of maple syrup urine disease At no time however a special odour was noted but the positive 2–4 dinitrophenylhydrazine reaction could have been consistent with this diagnosis Unidimensional paper chromatography of urine and blood aminoacids however did not show increased amounts of the branched-chain aminoacids

The normal initial paper chromatography of the blood aminoacids and the presence of a large aminoacid spot on the urinary chromatogram pointed to a no-threshold aminoaciduria The definite identification of this aminoacidopathy was finally made by column chromatography Three distinct ninhydrine positive peaks were found at the position of ASA and its B and C anhydrides These peaks overlapped with a standard solution of ASA added to a urine specimen of the patient On acidification of the urine the free ASA disappeared while the anhydride B and especially

of enzyme activity in our control human kidneys and in rats kidneys Furthermore in order to prove the validity of enzyme determinations in the stored frozen tissues arginase was assessed simultaneously with the argininosuccinase

## RESULTS

*Qualitative study*

Unidimensional paper chromatography of blood aminoacids was interpreted as normal On chromatography of the urine however a large very slowly running spot was found which usually is not revealed by this technique Several paper chromatographic procedures were employed in order to identify the abnormal urinary substance When ninhydrine staining was replaced by spraying Jaffe's reagent (1 % picric acid in 55 % ethylalcohol and 5 % potassiumhydroxyde in 85 % ethylalcohol) the substance stained as an orange spot Furthermore accentuation of this orange spot was obtained by acidifying the urine to pH 2 and boiling for 2 hours at 100°C

When the urine of the patient was brought to pH 10 and allowed to stand at room temperature for 48 hours this spot was only slightly visible With this procedure the excreted abnormal aminoacid behaved in the same manner as a standard solution of ASA Indeed in acidified urine free ASA is readily converted to the C anhydride which is easily perceptible on a chromatogram stained with picric acid KOH In alkaline urine however free ASA is mainly converted to its B anhydride which does not stain with picric and KOH This procedure appears to us as a valid screening technique for the identification of ASA

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*Quantitative study*

*Aminoacids* The concentrations of the urea cycle metabolites ammonia excepted in the

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| | Serum (µmol/l) | Urine (µmol/g nitrogen) | Liquor (µmol l) | Faeces (µmol 100 g) |
|---|---|---|---|---|
| Citrulline | 277 | 63 | 124 | 44 |
| Ornithine | 56 7 | 7 7 | 6 58 | 555 |
| Arginine | 40 3 | 5 3 | 13 8 | 16 6 |
| Free ASA | 3 45 | | 6 9 | |
| (B + C anhydride) | 551 | 425 | 724 | 168 |

different body fluids, faeces brain matter (18) and renal tissue are listed in Table 1 and 2

It appears from these data that ASA, which is absent in normal conditions, was present in appreciable amounts in all material examined The exceptionally high serum level was even exceeded by the liquor level The finding of ASA in the faeces was very surprising as this intermediate metabolite is not a usual nutrient constituent A plausible explanation may be that it originated from desquamated intestinal mucosal cells

Citrulline was also strikingly increased in the different body fluids and tissues, while arginine levels were interpreted as normal The serum and cerebrospinal fluid concentration of ornithine was decreased in comparison with an age matched control This compound was excreted in very high amounts in the faeces Immediate postmortem plasma levels of ASA and citrulline were 2240 and 84 µmoles per liter respectively

Table 2 *The concentration of urea cycle metabolites (ammonia excepted) in brain matter and renal tissue from the patient*

| | Brain matter (µmol/100 g wet tissue) | | | Renal medulla (µmol/100 g wet tissue) |
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| | Whole brain | White matter | Gray matter | |
| Citrulline | 44 2 | 24 7 | 44 | 12 4 |
| Ornithine | 20 4 | 25 5 | 20 4 | 13 7 |
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| ASA (B C anhydride) | 362 | 360 | 520 | 422 |

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|---|---|---|---|
| Control 1 | days | 77 5 | 1130 |
| Control 2 | 5 months | 114 1 | 900 |
| Control 3 | 1 days | 71 7 | 950 |
| Patient | 6 days | 0 5 | 1440 |

In the gray brain matter the argininosuccinase activity was estimated at about 2 7 of that found in the brain of normal controls (15) No argininosuccinase activity could be demonstrated in the patient's kidney tissue The arginase activity fell within the normal range or even exceeded slightly the mean controls (Table 4)

## DISCUSSION

An inborn error of metabolism was suspected in a four-days old child who progressively developed respiratory distress and cerebral involvement and who died at the age of six days The positive family history reinforced our suspicion a sibling of the proband died earlier of a similar disorder

Metabolic disorders with possible clinical symptoms in the first days of life and frequently with rapid fatal course are the maple syrup urine disease the hyperglycinaemia type I and II the isovaleric acidemia and to a lesser degree the galactosaemia

The clinical symptoms as presented by the patient could to a certain extent have been those of the classical form of maple syrup urine disease At no time however a special odour was noted but the positive 2–4 dinitrophenyl hydrazine reaction could have been consistent with this diagnosis Unidimensional paper chromatography of urine and blood aminoacids however did not show increased amounts of the branched-chain aminoacids

The normal initial paper chromatography of the blood aminoacids and the presence of a large aminoacid spot on the urinary chromatogram pointed to a no-threshold aminoaciduria The definite identification of this aminoacidopathy was finally made by column chromatography Three distinct ninhydrine positive peaks were found at the position of ASA and its B and C anhydrides These peaks overlapped with a standard solution of ASA added to a urine specimen of the patient. On acidification of the urine the free ASA disappeared while the anhydride B and especially

of enzyme activity in our control human kidneys and in rats kidneys Furthermore in order to prove the validity of enzyme determinations in the stored frozen tissues arginase was assessed simultaneously with the argininosuccinase

## RESULTS

*Qualitative study*

Unidimensional paper chromatography of blood aminoacids was interpreted as normal On chromatography of the urine, however a large very slowly running spot was found which usually is not revealed by this technique Several paper chromatographic procedures were employed in order to identify the abnormal urinary substance When ninhydrine staining was replaced by spraying Jaffe s reagent (1% picric acid in 55 ethylalcohol and 5% potassiumhydroxyde in 85% ethylalcohol) the substance stained as an orange spot Furthermore accentuation of this orange spot was obtained by acidifying the urine to pH 2 and boiling for 2 hours at 100°C

When the urine of the patient was brought to pH 10 and allowed to stand at room temperature for 48 hours this spot was only slightly visible With this procedure the excreted abnormal aminoacid behaved in the same manner as a standard solution of ASA Indeed, in acidified urine free ASA is readily converted to the C anhydride which is easily perceptible on a chromatogram stained with picric acid KOH In alkaline urine however free ASA is mainly converted to its B anhydride which does not stain with picric acid KOH This procedure appears to us as a valid screening technique for the identification of ASA

Unequivocal identification of the aminoacid was finally made by column chromatography

Unidimensional paper chromatography of the aminoacids in brain tissue did not reveal the presence of ASA

*Quantitative study*

*Aminoacids* The concentrations of the urea cycle metabolites ammonia excepted in the

Table 1 *The concentration of the urea cycle metabolites (ammonia excepted) in serum urine cerebrospinal fluid and faeces from the patient*

| | Serum (µmol/l) | Urine (µmol/g nitrogen) | Liquor (µmol/l) | Faeces (µmol/10 |
|---|---|---|---|---|
| Citrulline | 277 | 63 | 124 | 44 |
| Ornithine | 56 7 | 7 7 | 6 58 | 555 |
| Arginine | 40 3 | 5 3 | 13 8 | 16 6 |
| Free ASA | 3 45 | | 6 9 | |
| (B+C anhydride) | 551 | 425 | 724 | 168 |

different body fluids faeces brain matter (18) and renal tissue are listed in Table 1 and 2

It appears from these data that ASA, which is absent in normal conditions, was present in appreciable amounts in all material examined The exceptionally high serum level was even exceeded by the liquor level The finding of ASA in the faeces was very surprising as this intermediate metabolite is not a usual nutrient constituent A plausible explanation may be that it originated from desquamated intestinal mucosal cells

Citrulline was also strikingly increased in the different body fluids and tissues, while arginine levels were interpreted as normal The serum and crebrospinal fluid concentration of ornithine was decreased in comparison with an age matched control This compound was excreted in very high amounts in the faeces Immediate postmortem plasma levels of ASA and citrulline were 2240 and 84 µmoles per liter respectively

Table 2 *The concentration of urea cycle metabolites (ammonia excepted) in brain matter and renal tissue from the patient*

| | Brain matter (µmol/100 g wet tissue) | | | Renal medulla (µmol/100 g wet tissue) |
|---|---|---|---|---|
| | Whole brain | White matter | Gray matter | |
| Citrulline | 44 2 | 24 7 | 44 | 12 4 |
| Ornithine | 20 4 | 25 5 | 20 4 | 33 7 |
| Arginine | | 34 5 | 32 2 | 27 4 |
| ASA (B+C anhydride) | 362 | 360 | 520 | 422 |

tivity could be demonstrated in our patient while this enzymatic activity was shown to be normal in the case reported by Baumgartner (4)

## SUMMARY AND CONCLUSIONS

A second case of neonatal death due to argininosuccinic aciduria is described The first clinical symptoms of this particularly malignant variant appeared at the age of four days Death occurred at the age of 6 days The clinical picture consisted of rapidly increasing apathy and respiratory distress generalized hypotonia myoclonic seizures and terminal oligouria The biochemical investigations mainly revealed an elevated concentration of ASA and citrulline in the urine blood and cerebrospinal fluid The amounts of ASA in brain and renal tissue and in the faeces were also increased this has never been described before A deficient argininosuccinase activity was demonstrated in the blood cells and in brain and kidney tissue

The neonatal variant of argininosuccinic aciduria should be distinguished from the classically described form with later onset of the clinical symptoms

Both variants are thought to be another example of genetic heterogeneity A review of the family histories of the patients with classical argininosuccinic aciduria did not reveal possible cases of the neonatal variant A sibling of the proband died from a similar disease at the age of 5 days

## ADDITIONAL NOTE

When this paper was finished another male child was born in this family The newborn was immediately transferred to our department for an enzymatic assay The argininosuccinase activity in blood cells was 13 µmoles of urea formed per hour per gram haemoglobin which is consistent with the heterozygous state of the condition. The parents could be reassured within 4 hours and breastfeeding could be started The infant was reexamined at the age of 10 days and 11 weeks. Major problems have not arisen Clinical examination was normal The mental and motoric progress was as satisfactory as possible No argininosuccinic acid could be detected in the urine specimens In this way the importance of enzymatic assays in the immediate neonatal period is stressed

## ACKNOWLEDGEMENTS

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the C anhydride increased Elevated ASA concentrations were found in the urine blood liquor stools brain and tissue of the patient, while its immediate precursor citrulline was increased in the urine, blood liquor and in the brain and renal tissue A deficient argininosuccinase activity was demonstrated in the patient s blood cells brain and kidney tissue Both parents and their healthy daughter were shown to be heterozygotes

Up to now and including our patient the diagnosis of argininosuccinic aciduria was biochemically confirmed in 16 patients from 11 families Three more children who were not examined, were very probably affected by the same disorder as they presented a similar clinical picture as their sibling in whom the diagnosis was established (3 4 7 9 16 20 23 26)

The clinical picture of argininosuccinic aciduria in its common form is dominated by a generally very pronounced mental retardation Developmental milestones are reached slowly or not at all Periods of coma or ataxia may intervene Hepatomegaly may be seen in some patients Frequently hairs and nails are brittle and frail The trichopathy is usually described by dermatologists as trichorrhexis nodosa These features were present in the first two reported patients detected by Allan *et al* (2) and more or less completely noticed in 12 other patients

The clinical picture as presented by the proposita, differs clearly from the classical form mainly by the very early onset of the symptoms and by the fulminant and fatal course That this was due to chance is very improbable as a sibling died previously after a very similar disease When this paper was in preparation Baumgartner *et al* reported a similar case of neonatal death due to argininosuccinic aciduria (4) Earlier only Moser *et al* (22) and especially Levin *et al* (16) mentioned temporary neonatal difficulties, in various degrees in patients with argininosuccinic aciduria Later on, the classically described clinical picture was present

The prominent clinical symptoms in Baumgartner s patient were poor sucking apathy and muscular hypotonia, increasing respiratory distress and seizures The first symptoms appeared on the third day of life Death occurred at the age of nine days The ASA serum level was 392 μmoles per liter The 2–4 dinitrophenylhydrazine test in the urine was positive as it was in our patient This finding remains unexplained Indeed, the urinary excretion of α ketoacids was demonstrated to be normal in the proposita Baumgartner also mentioned a strongly positive cyanide nitroprusside test

Both the clinical picture and the biochemical abnormalities are largely different from the findings in the classically described form of argininosuccinic aciduria In the latter variant, a positive 2–4 dinitrophenylhydrazine reaction has hitherto never been mentioned Furthermore ASA reaches three to four times lower blood levels than in the neonatal variant A relatively higher protein supply in the neonatal period cannot be taken in account as breast feeding during 48 hours could already provoke an irreversible condition

It is well known that an intercurrent infection can provoke a metabolic decompensation through an increased catabolism This phenomenon has been reported in phenylketonuria (5) intermittent ketoaciduria (11) isovaleric acidemia (6) and was observed by ourselves in patients with homocystinuria and cystathioninuria (13) Pulmonary complications as found at autopsy in the neonatal form are supposed to be only secondary to the cerebral involvement

It is much more likely that the variation in clinical and biochemical features in both forms of argininosuccinic aciduria is just another example of genetic heterogeneity

One could speculate that in the neonatal variant the enzymatic defect is quantitatively more pronounced than in the classical form by a different alteration in the argininosuccinase protein The question may even be put forward if both cases of neonatal argininosuccinic aciduria are not genetically different subtypes Indeed no renal argininosuccinase ac

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CASE REPORT

# INTRAVASCULAR COAGULATION IN GENERALIZED HERPES SIMPLEX INFECTION OF THE NEWBORN

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In the newborn a hemorrhagic tendency has been associated with disseminated herpes simplex infection (1 9) Although thrombocytopenia and hypoprothrombinemia have been recognized extensive coagulation studies apparently have not been performed and the pathogenesis of the bleeding diathesis remains obscure (3 16 17 19) Since hemorrhage is a prominent feature of this disease it would be useful to clarify the nature of the underlying mechanism responsible for the abnormal bleeding so that appropriate therapy could be administered

The present report describes the virological hematological and pathological findings in a case of disseminated herpes simplex infection in a newborn associated with a fatal hemorrhagic diathesis

## CASE REPORT

J B was admitted to Childrens Hospital of Los Angeles at 5 days of age His 1 year-old mother had been well until 8 months gestation when she developed fever to 41 C No vaginal irritation or herpetic lesions were noted. Five days later her membranes ruptured spontaneously and labor began At birth the infant appeared normal and weighed 2.5 kg He was discharged at days of age On the fifth day of life he developed diarrhea rhinorrhea cough, and tachypnea He was admitted to the hospital with pneumonia and possible sepsis

Physical examination revealed the following weight 2 2 kg pulse 160/min respirations 90/min and temperature 37 6 C The patient was 10 dehydrated and lethargic Bilateral fine rales were heard in the chest The liver was palpable cm below the right costal margin but no icterus was noted The spleen was not palpable and there were no focal neurologic signs or skin lesions The patient was placed in oxygen and mist

A lumbar puncture was performed and the cerebrospinal fluid (CSF) contained 1 WBC and 1 RBC per mm 35 mg/100 ml sugar and 65 mg/100 ml protein Routine bacteriological cultures of CSF urine and blood revealed no organisms Hemoglobin was 12 7 g/100 ml PCV 40 % WBC 12 300 per mm with 40 polymorphonuclear leukocytes 52 lymphocytes and 8 monocytes slight anisocytosis and poikilocytosis were noted Adequate numbers of platelets were present on the peripheral smear a sickle cell preparation was negative Blood chemistries included a blood sugar of 65 mg/100 ml BUN of 3 mg/100 ml sodium of 147 mEq/l potassium of 5 0 mEq/l chloride of 104 mEq/l and CO of 28 mEq/l Roentgenogram of the chest revealed bilateral infiltrates Intravenous penicillin (100 000 units/kg/day) and intramuscular kanamycin (15 mg/kg day) were administered

On the second hospital day the patient was still tachypneic and a repeat roentgenogram of the chest revealed progression of the pulmonary disease The infant developed respiratory fatigue and required an Engstrom respirator Intravenous penicillin was discontinued and intravenous methicillin (200 mg/kg/day) was begun Six hours later blood was seen oozing from venipuncture sites and throat secretions became blood tinged Hemoglobin was 8 0 g/100 ml PCV 25 % platelet count 45 000 per mm and the Quick prothrombin time over 40 seconds. Vitamin K oxide (1 0 mg intramuscularly) was given and the patient was transfused with sedimented red cells (10 cc kg) and fresh frozen plasma (10 cc/kg) Be

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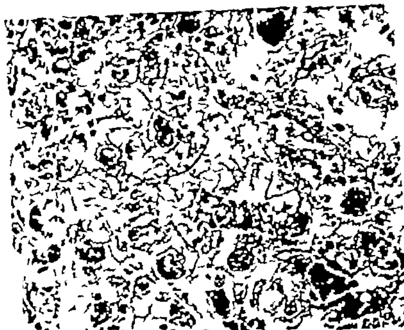


Fig. 2. Liver showing fibrin deposition (black dots) between necrotic hepatocytes which show loss of cytoplasmic detail, description of cell membranes and nuclear degeneration. The liver cord pattern is obscured by swelling of the necrotic liver cells. Phosphotungstic acid hematoxylin stain. Approx. 500.

the pharynx, trachea, upper esophagus, kidney and adrenal cortex. The liver was enlarged, soft and mottled red and yellow. There was focal subarachnoid hemorrhage. The brain was soft and congested and had petechial hemorrhages on the cut surfaces.

Microscopically, foci of necrosis in the lungs were circumscribed by hemorrhage. Sequestration of platelets and fibrin in lumina of alveoli was disclosed by phosphotungstic acid haematoxylin stains (Fig. 1). Intravascular coagulation was also present in and near the necrotic foci. In addition, there was generalized interstitial pneumonitis with numerous cells containing intranuclear inclusions.

Extensive destruction of liver parenchyma by focal necrosis was associated with many intranuclear inclusions. Fibrin and platelet deposition was present in virtually all intact sinusoids (Fig. 2). Foci of herpetic necrosis in the adrenal cortex were small but inclusions were present.

The bone marrow was normal; megakaryocytes were plentiful. Although cerebral edema and congestion were present, no inclusion bearing cells were identified.

## VIROLOGY STUDIES

*Methods.* The possibility of a viral infection was not considered prior to death of this infant, therefore no pre-mortem specimens were available for viral cultures.

At autopsy, specimens of adrenal gland, esophagus, large intestines, small intestines, kidney, liver, lung and myocardium were obtained for viral studies. Twenty per cent suspensions of the tissues were made in Hank's balanced saline solution containing penicillin and streptomycin. Suspensions were centrifuged for 10 minutes at 2500 r.p.m. in a refrigerated International PR type 2 centrifuge. The supernatant fluids were carefully removed and treated with penicillin (1000 units/ml) and streptomycin (1000 ug/ml) at room temperature for one hour.

Two-tenths milliliter aliquots of each specimen were inoculated into each of 4 cell cultures of human embryonic kidney (HEK) cell cultures containing Eagles medium supplemented with 2 % inactivated calf serum (18). The cell cultures were examined daily for cytopathic effect (CPE).

Identification of isolated agents was accomplished by combining equal amounts (0.3 ml) of virus (100

Table 1 *Coagulation studies*

| | Hospital day | | |
|---|---|---|---|
| | 2 | 3 | 4 |
| Platelet count mm³ (normal 150 300 000) | 45 000 | 30 000 | 25 000 |
| *Quick prothrombin time* (control 12 sec) | 40 sec | 84 sec | — |
| Activated partial thromboplastin time (control 43 sec) | — | 130 sec | — |
| Thrombin time (control 15 sec) | — | 77 sec | — |
| *Fibrinogen mg/100 ml (normal 200–400)* | — | 78 | 48 |
| Factor V (normal 50–150 ) | — | 12 | — |
| Factor VIII (normal 50–150 ) | — | 80 | — |
| Factor IX (normal 50–150 ) | — | 85 | — |
| Euglobin lysis time (normal greater than 2 hs) | — | Greater than 2 hours | — |
| Fibrin split products (normal–absent) | — | Absent | — |

*cause of continued bleeding the possibility of intra*vascular coagulation was considered and further coagulation studies were performed (Table 1) Intravenous *heparin therapy was administered at the rate of* 100 units/kg/every 4 hours No clinical improvement was noted The patient developed seizures ir reversible hypotension and subsequently expired 18 hours after beginning heparin therapy No history suggesting infection with herpes simplex virus could be obtained from the immediate family or from the hospital nursery personnel

## METHODS

Platelet counts were performed by the method of Brecher & Cronkite (2) Prothrombin time was done by the method of Quick *et al* (13) kaolin partial thromboplastin time by the method of Proctor & Rapaport (12) and thrombin time as outlined by Hardisty & Ingram (7) Plasma fibrinogen was measured by the technique of Jacobsson (8) Factor V *by the Stormorken method (15)* and Factors VIII and IX were determined by the one stage method based on the activated partial thromboplastin time (12) Euglobin lysis was measured as outlined by Fletcher *et al* (6) Fibrin split products were estimated in the patient's serum after the addition of thrombin to the serum Immunoelectrophoresis was *performed in a 1 agar gel against fibrinogen* and serum (Hyland Laboratories Los Angeles Calif)

## PATHOLOGY

At necropsy the patient was not grossly icteric Focal superficial hemorrhages were present in

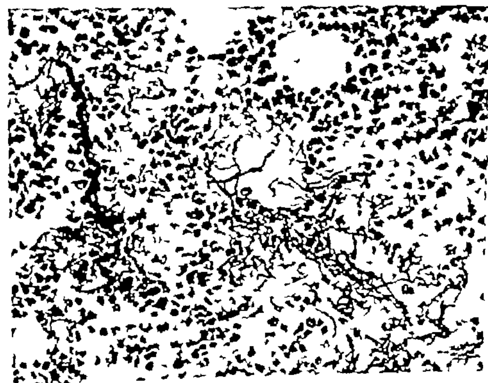


*Fig 1* Lung showing fibrin deposition and hemorrhage near a *focus of* herpetic necrosis The fibrin appears as black strands of variable length and diameter Necrosis is most marked in the lower center *of the field* Intranuclear inclusions are not discernible at this magnification *Phosphotungstic acid hematoxylin stain* 250

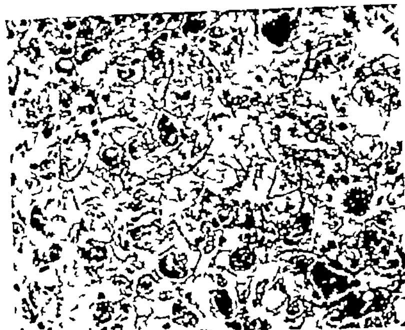


Fig 2 Liver showing fibrin deposition (black trash) between necrotic hepatocytes which show loss of cytoplasmic detail description of cell membranes and nuclear degeneration The liver cord pattern is obscured by swelling of the necrotic liver cells Phosphotungstic acid hematoxylin stain Approx 500

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Two-tenths milliliter aliquots of each specimen were inoculated into each of 4 cell cultures of human embryonic kidney (HEK) cell cultures containing Eagles medium supplemented with 2% inactivated calf serum (18) The cell cultures were examined daily for cytopathic effect (CPE)

Identification of isolated agents was accomplished by combining equal amounts (0 3 ml) of virus (100

TCID ) with 1 10 dilution of herpes simplex anti sera and incubating these mixtures at room temperature for 1 hour before inoculating them into replicate cell cultures Herpes simplex hyperimmune guinea pig serum with a C F titer of 1 128 was obtained from the Department of Health Education and Welfare Public Health Service Communicable Disease Center Biological Reagents Section Atlanta Ga

*Results* Within 48 to 72 hours after primary inoculation, all specimens (adrenal gland esophagus large intestine small intestine, kidney liver lung and myocardium) caused cytopathic effect in tissue culture characterized by rounding and increased refractibility of the cells this progressed to disintegration of tissue culture Seven days after inoculation supernatant fluid was passed to fresh HEK cell cultures and cytopathic effect was again observed

In the virus identification tests within 4 days after inoculation virus controls showed cytopathic effect which progressed to disintegration of tissue culture Cell cultures inoculated with the virus antiserum mixtures showed no cytopathic effect when last observed one week after inoculation indicating neutralization of the virus by the herpes simplex antiserum

## DISCUSSION

A bleeding tendency has been observed in 20 of the 54 cases of disseminated herpes simplex infections of the newborn reported in the literature (1 9) Thrombocytopenia was documented in three reports (3 16 17) Hypoprothrombinemia secondary to hepatic failure has been proposed as the mechanism of this bleeding disorder (19) but to our knowledge no extensive coagulation studies have been reported

In this patient extensive hepatic necrosis could have resulted in prolongation of the prothrombin time, and decreased levels of fibrinogen and Factor V However the absence of jaundice suggests that liver dysfunction alone was not sufficiently severe to cause this degree of coagulation factor depletion In addition the level of Factor IX a vitamin K dependent factor frequently reduced in liver disease (14) was normal in this patient Thrombocytopenia could not be adequately explained on the basis of liver failure alone Although infection can cause thrombocytopenia presumably through bone marrow depression the presence of adequate numbers of megakaryocytes in the bone marrow of this patient suggests increased peripheral utilization of platelets as the cause of the thrombocytopenia

It is generally accepted that thrombocytopenia fibrinogenopenia and reduced levels of Factors V and VIII are the classical findings in consumptive coagulopathies (10) This patient exhibited all of these findings with the exception of Factor VIII depletion The autopsy findings of extensive platelet and fibrin deposition in the liver and lungs establish that both intravascular and extravascular coagulation had taken place It is interesting to speculate that in this situation local tissue necrosis caused the release of thromboplastin like substances which could have resulted in this deposition of fibrin Normal to increased levels of Factor VIII have been reported by others in the presence of laboratory and histological evidence of intravascular coagulation (4 5) This may represent a compensatory increase of Factor VIII as a result of stress

Intravascular coagulation has been documented as an intermediary mechanism in various disease states (10) It has been recognized in association with certain viral infections including varicella vaccinia variola rubella rubeola and arboviruses causing hemorrhagic fevers (11) It now appears that herpes simplex virus may also provoke this phenomenon

The findings in the present patient suggest that intravascular coagulation plays a role in the hemorrhagic diathesis in herpes simplex infection of the newborn Adequate coagulation studies on such patients should be performed in order to provide a rational basis for therapy since anticoagulants particularly heparin may be indicated The indiscriminate use of plasma and/or platelet transfusions in the absence of adequate heparin therapy could aggravate the

situation by providing increased substrate for intravascular clotting. More extensive studies on this group of patients seem indicated since fatal hemorrhage may complicate herpes simplex infection of the newborn.

## SUMMARY

A case of disseminated herpes simplex virus infection in a newborn associated with a fatal bleeding diathesis is reported. The presence of intravascular coagulation was suggested by thrombocytopenia, fibrinogenopenia and reduced Factor V level. At necropsy localized fibrin and platelet deposition was found in liver and lung. It is postulated that intravascular coagulation resulted from local tissue necrosis and the subsequent release of thromboplastin like substances into the general circulation. Heparin therapy did not alter the fatal outcome.

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*Key words:* Neonate, herpes simplex, intravascular coagulation

TCID$_{50}$) with 1 10 dilution of herpes simplex antisera and incubating these mixtures at room temperature for 1 hour before inoculating them into replicate cell cultures Herpes simplex hyperimmune guinea pig serum with a C F titer of 1 128 was obtained from the Department of Health Education and Welfare Public Health Service Communicable Disease Center Biological Reagents Section Atlanta Ga

*Results* Within 48 to 72 hours after primary inoculation all *specimens (adrenal gland* esophagus, large intestine small intestine kidney liver lung and myocardium) caused cytopathic effect in tissue culture characterized by rounding and increased refractibility of the cells *this progressed to disintegration of tissue* culture Seven days after inoculation supernatant fluid was passed to fresh HEK cell cultures and cytopathic effect was again observed

In the virus identification tests within 4 days *after inoculation virus controls showed* cytopathic effect which progressed to disintegration of tissue culture Cell cultures inoculated with the virus antiserum mixtures showed no cytopathic effect when last observed one week *after inoculation indicating neutraliza*tion of the virus by the herpes simplex antiserum

## DISCUSSION

A bleeding tendency has been observed in 20 of the 54 cases of disseminated herpes simplex infections of the newborn reported in the literature (1 9) Thrombocytopenia was docu*mented in three reports* (3 16 17) Hypoprothrombinemia secondary to hepatic failure has been proposed as the mechanism of this bleeding disorder (19) but to our knowledge no extensive coagulation studies have been re*ported*

In this patient extensive hepatic necrosis could have resulted in prolongation of the prothrombin time, and decreased *levels of fibrino*gen and Factor V However the absence of jaundice suggests that liver dysfunction alone was not sufficiently severe to cause this degree of coagulation factor depletion In addition the level of Factor IX a vitamin K dependent factor frequently reduced in liver disease (14) was normal in this patient Thrombocytopenia could not be adequately explained on the basis of liver failure alone Although infection can cause thrombocytopenia presumably through bone marrow depression the presence of adequate numbers of megakaryocytes in the bone marrow of this patient suggests increased peripheral utilization of platelets as the cause of the thrombocytopenia

It is generally accepted that thrombocytopenia fibrinogenopenia and reduced levels of Factors V and VIII are the classical findings in consumptive coagulopathies (10) This patient exhibited all of these findings with the exception of Factor VIII depletion The autopsy findings of extensive platelet and fibrin deposition in the liver and lungs establish that both intravascular and extravascular coagulation had taken place It is interesting to speculate that in this situation local tissue necrosis caused the release of thromboplastin like substances which could have resulted in this de*position of fibrin Normal to increased levels of* Factor VIII have been reported by others in the presence of laboratory and histological evidence of intravascular coagulation (4 5) This may represent a compensatory increase of Fac*tor VIII as a result of stress*

Intravascular coagulation has been documented as an intermediary mechanism in various disease states (10) It has been recognized in association with certain viral infections including varicella vaccinia variola rubella rubeola and arboviruses causing hemorrhagic fevers (11) It now appears that herpes simplex virus may also provoke this *phenomenon*

The findings in the present patient suggest that intravascular coagulation plays a role in the hemorrhagic diathesis in herpes simplex infection of the newborn Adequate coagulation *studies on such patients should* be performed in order to provide a rational basis for therapy since anticoagulants particularly heparin may be indicated The indiscriminate use of plasma and/or platelet transfusions in the absence of adequate heparin therapy could aggravate the

situation by providing increased substrate for intrava ular clotting More extensive studies on this group of patients seem indicated since fatal hemorrhage may complicate herpes sim plex infection of the newborn

## SUMMARY

A case of disseminated herpes simplex virus infection in a newborn associated with a fatal bleeding diathesis is reported The presence of intravascular coagulation was suggested by thrombocytopenia fibrinogenopenia and re duced Factor V level At necropsy localized fibrin and platelet deposition was found in liver and lung It is postulated that intravas cular coagulation resulted from local tissue ne crosis and the subsequent release of thrombo-plastin like substances into the general circula tion Heparin therapy did not alter the fatal outcome

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# PROCEEDINGS OF PAEDIATRIC SOCIETIES

## DANISH PAEDIATRIC SOCIETY

### Meeting April 17 1968

B Biering Sorensen Vagn Christensen Chr Hansted Ole Mortensen & Poul Vogensen *Recent experience in prophylactic investigation of the health and welfare of pre school children* (To be published in Månedsskrift for praktisk lægegerning)

### Meeting May 8 1968

J P M Tizard (Institute of Child Health Hammersmith Hospital University of London) *Perinatal illnesses and chronic brain damage*

### Meeting Sept 18 1968

J E Olsen *Congenital non spherocytic haemolytic anaemia as the result of glucose 6 phosphate dehydrogenase deficiency in a Danish infant*

A case of neonatal icterus and haemolytic anaemia in a male infant is reported Pregnancy and delivery had been uncomplicated Five days prior to the delivery the mother received 20 mg menadion bisulphite (soluble vitamin K) orally

The infant developed jaundice one hour after birth Ten hours after birth the serum bilirubin was found to be 7 8 mg and entirely of indirect type The serum bilirubin concentration attained a maximum on the fourth day of life when the total serum bilirubin was 16 4 mg %. On the second day of life the haemoglobin (Sicca) was 100 . The jaundice disappeared in the course of a few weeks Coombs test and dextran reaction were negative The patient s blood type and that of his mother were 0 Rhesus positive

At the age of one month the patient was admitted to the local hospital in an emaciated condition Examination of the blood showed a haemoglobin concentration of 5 2 g MCV 108 mµl MCHC 31 g reticulocytes 14–22 ‰ Icteric index 8 Haptoglobin 24 mg Examination of bone marrow revealed findings as in haemolytic anaemia No abnormal cell forms were encountered

At the age of 2½ months the infant was transferred to the Paediatric Department Arhus Municipal Hospital On admission the liver and spleen were found to be palpable 1½ cm below the costal margin By and large examination of the blood revealed unchanged conditions Haptoglobin was 112 mg As non spherocytic anaemia was suspected the following investigations were conducted by Dr Esper Mortensen leader of the Central Laboratory Osmotic resistance was normal but could be reduced to a normal extent only on incubation for 24 hours at 37°C The concentration of gluthation was normal but was re

duced by incubation with menadion ATP concentration and pyruvate kinase activity were normal Glucose 6-phosphate-dehydrogenase activity was markedly reduced At two investigations activities of 0 5 and 0 72 mic mmol/min × g Hb were encountered the normal range being 6-10 Electrophoresis of haemoglobin yielded normal results

Examination of the patient's parents and sister revealed normal glucose 6-phosphate dehydrogenase activities The family was of Danish origin and there was no known predisposition to jaundice or anaemia

The patient is thriving and at the age of nine months his haemoglobin concentration is risen to approximately 9 g

Glucose 6-phosphate-dehydrogenase deficiency is encountered extensively among negroes and among the white population in the Mediterranean regions but is very rare north of the Alps Isolated cases have been recognised in the north

Discussion

*F Vesterdal* Is haemolytic anaemia following consumption of horse beans identical?

*J E Olsen* Yes but favism can occur without deficiency of glucose 6-phosphate-dehydrogenase

Karsten Rasmussen *Excretion of phosphoryl ethanolamine in hypophosphatasia and in normal individuals*

Phosphorylethanolaminuria was first described in 1955 in patients with hypophosphatasia while phosphorylethanolamine (EAP) has not previously been identified in the urine from normal individuals

Twenty four hour specimens of urine from a normal material comprising 54 children and 12 adults aged from 18 days to 44 years were analysed for EAP by means of a specific sensitive column chromatographic technique All of the individuals investigated excreted EAP in the urine

The excretion diminishes from 62 0 ± 20 0 μmol per m body surface per 24 hours during the first four years of life to 18 2 ± 6 1 μmol in adults This is independent of sex food intake or the time of day

In five heterozygotic carriers for hypophosphatasia (2½ –63 years) the excretions were found to be from 2 7 to 7 6 times the normal excretion in the age groups concerned

Patients with hypophosphatasia excrete from 10 to 47 times the normal quantity (eight determinations in two patients aged four and 35 years)

Short term determinations of renal clearance for endogenic EAP in fasting control individuals during water loading tests revealed definite differences between the three following groups

In six normal adults the renal clearance in a total of nine determinations was on an average 8 0 ml per minute per 1 73 m body surface which is less than 10 of the creatinine clearance In three adult carriers for hypophosphatasia the renal EAP clearance was on an average 29 ml/min per 1 73 m body surface (21–38) in a total of seven determinations = 18–48 of the creatinine clearance In the adult patient with hypophosphatasia the renal EAP clearance was found to be 130 76 and 120 at three separate determinations or 98 62 and 92 % of the creatinine clearance

The conclusion from this is that hyperphosphorylethanolaminuria in hypophosphatasia is of renal type This finding compared with the demonstrated increase in EAP concentration in the plasma in hypophosphatasia suggests a generalised transport defect for EAP through the cell membrane in hypophosphatasia This presumption is supported by the flat EAP disappearing curves in plasma in hypophosphatasia following intravenous loading with EAP in contrast to the steep curves in normal individuals Frequent determinations of inorganic phosphate ethanolamine and EAP in urine and in plasma during loading tests with EAP re

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renal cortex The disease is very rare in child hood

It appear to be reasonable to differentiate between the following types of the disease A The idiopathic (cryptogenic) type B Autoim mune polyglandular type (at present the most common type in adults) C Cases following infections D The Addison Schilder syndrome (combination with diffuse cerebral sclerosis) E Aplasia of the adrenal cortex F Cases with recognised lesions of the adrenal cortex such as tumours cysts and traumatic haemorrhage

The authors patient was admitted to Sundby Hospital on account of persistent vomiting He was found to have very low plasma sodium values and very high plasma potassium values ...e t value were normalised on a high so... ...m low potassium diet Subsequently die... ...y treatment was replaced by administration 0.1 mg Florinef[R] (Squibb) and 15 mg cor...one acetate daily He appears to be able to ...d a normal life on this therapy

The diagnosis was confirmed by determination of a low aldosterone secretion rate very low plasma cortisol values and a low 17 KGS excretion On stimulation with synactene no responses occurred in the plasma cortisol values or the 17 KOS excretion Further the patient was shown to have very high serum ACTH values

On admission he was very tanned although it was midwinter This pigmentation has since disappeared on therapy with corticoids

Examination for adrenocortical antibodies yielded negative results and there was no evidence of other endocrine diseases No infections were suspected as being responsible for the disease

The EEG findings and neurological examination gave no evidence to support the diagnosis of the Addison Schilder syndrome and thus the patient is considered as most probably belonging to the idiopathic group

## Meeting Oct 9 1968

... Oster *Rare diseases from Randers*

...emonstration of the following syndromes

1. Macroglossia–omphalocele (Beckwith Wiedemann)
2. Pectoral muscle aplasia–syndactyly (Poland)
3. Osteochondrosis deformans of the tibia (Blount)
4. Epimetaphyseal changes (Turner)
5. Hemimelia–thrombopenia–anaemia with leukaemoid reaction (Schonenberg Degnan)
6. Pancreatic insufficiency–neutropenia (Schwachman)
7. Adhesions of the labia minora
8. Klinefelter s syndrome

J Oster *Red naevus on the nuchal region and telangiectasis in the upper interscapular region*

An investigation of the incidence of the above mentioned phenomena their significance and their relationship to other skin diseases (To be published elsewhere)

C J Ingomar *Fat excretion in infants with prolonged dyspepsia*

A material of 133 children with prolonged dyspepsia was investigated in view of steatorrhoea In 27 the average daily excretion was found to be >7 g and the most frequent cause of this was cystic fibrosis of the pancreas and gluten intolerance The greatest excretion of fat was found in underweight children with abnormal stools receiving normal diets Abnormal d xylose tolerance and radiological evidence of enteritis were found in approximately 40 and approx 30 % respectively of the children with fat excretion of >7 g per 24

veal that this difference in the form of the curve cannot be explained solely by greater hydrolytic splitting of EAP extracellularly in normal individuals

*Discussion*

*Torben Iversen* Demonstration by means of slides of the patients mentioned and another case

*J Vesterdal* What is the excretion of EAP in conditions with raised phosphatases?

*K Rasmussen* We found normal EAP excretion in a boy aged 15 years with raised phosphatase values (osteogenic sarcoma) and low EAP excretion was encountered in three children with rickets

*B Friis Hansen* Are raised values for EAP excretion found in other forms of amino acid uria?

*K Rasmussen* No

H Agerbaek & J E Olsen *Extremely high protein bound iodine values in three healthy siblings*

The iodine containing radio opaque substance for biliary investigation Tendax or alpha ethyl beta (2 4 6 triiodo-3 hydroxyphenyl) propionic acid which was employed in the nineteen fifties can still give rise to diagnostic difficulties in thyroid disease

PBI values of approximately 50 microgram % were encountered in 1967 in three clinically healthy siblings whose mother had been submitted to radiography after administration of Tendax ten years previously and six months prior to the birth of the first child This substance has a half life in the serum of a couple of years and it is known that in pregnancy Tendax is transferred to the foetus via the placenta The youngest child was born with goitre which disappeared spontaneously during the first months of life The goitre was possibly due to the fact that the mother had received Neomercazol (Schering) (= Carbimazolum NFN) in the first two months of the pregnancy The iodine metabolism in this child was thoroughly investigated when she was 2 1/ years old PBI was then 52 microgram Thyroid function was normal as assessed by the following measurements serum cholesterol T 3 ephadex uptake TBG capacity I[1] and total iodine uptake in the thyroid gland PBI[1] concentration serum thyroxin and the half life of thyroxin in the serum Serum chromatography in three different systems after administration of I[1] showed radioactivity corresponding to the thyroxine bands only and serum electrophoresis showed that the radioactivity was bound in a normal manner to the various protein fractions The greatly raised PBI was found to be electively associated with the serum albumin Iodine excretions in the urine were 19 20 and 27 microgram per 24 hours

The finding of perfectly normal function of the thyroid gland and the normal iodine excretion in the urine confirm Tendax unique binding in the serum

*Discussion*

*C Hanstead* Does this hold true for Tendax only?

*H Agerbaek* This is the only preparation which has such a long half life

*T Jersild* After administration of 500 mg iodchloroxychinoline to a mother during delivery we found raised PBI in both mother and infant after delivery

I Møller & E B Buch *A case of Addison's disease in a boy aged ten years*

According to modern diagnostic procedures Addison's disease is defined as the symptom complex produced by chronic generalised insufficiency of adrenocortical hormonal secretion resulting from a primary lesion of the ad

Fleming, Guttler Erling S Olesen & Erik Wamberg *The influence of phenylalanine free diet on the diurnal variations in serum phenylalanine and tyrosine in children with phenylketonuria*

In his first report on imbecillitas phenylpyruvica in 1934 Asbjorn Folling realised that the disease was due to a hereditary defect in phenylalanine metabolism The prophylactic and therapeutic consequences of this observation have first become apparent in the past 15 years

During recent years it has proved possible to assess the results of treatment Reliable assessment requires a comparable control material certain diagnosis and treatment which is optimal in all respects

In a brief review it is explained why investigation of the urine of newly born infants for excretion of ketone bodies can no longer be considered adequate In the John F Kennedy Institute two fluorometric methods for determination of serum phenylalanine and tyrosine are employed For this double determination ml blood is required Further the excretion o hydroxyphenyl acetic acid and p hydroxy henyl pyrodruvic acid in the urine are determined Establishement of the diagnosis of classical phenylketonuria requires fasting serum phenylalanine of over 30 mg/100 ml 1800 μmol/l) phenylalanine tyrosine ratio of over 10 abnormal phenylalanine tolerance urve defective excretion of p-hydroxyphenyl pyrodruvic acid S rum phenylalanine values between 10 and 30 mg/100 ml (610–1800 μmol l) require special diagnostic considerations including extensive investigation of amino acid metabolism The therapeutic possibilities in this group are mentioned in the subsequent contribution Excretion of p hydroxyphenyl pyrodruvic acid in connection with a low phenylalanine tyrosine ratio suggest a defect in tyrosine metabolism

Assessment of the influence of the phenylalanine reducing diet on the serum phenylalanine level requires well established criteria for the times of the day at which the blood samples should be withdrawn Our investigations have shown that serum phenylalanine concentrations in children with phenylketonuria receiving treatment are highest in the morning before the first meal and lowe t at about midnight The diurnal variations in serum tyrosine are the opposite of this This holds true also for the diurnal variations in untreated children with phenylketonuria and normal individuals The fall in serum phenylalanine concentration in children with phenylketonuria treated with diet is approximately 1–1 ¹/ mg/100 ml from 7 a m to 7 p m If the dietary treatment is regulated according to the phenylalanine concentration in blood samples taken in the fasting state evening values which are not too low must be ensured in the choice of a therapeutic level The fasting morning level should scarcely be lower than 4–7 mg/100 ml

The decrease in the serum phenylalanine concentration observed in the course of the day in children with phenylketonuria treated with diet depends upon administration of the phenylalanine free protein hydrolysate Albumaid® (Scientific Hospital Supply Liverpool) Fasting or calorie intake in the form of carbohydrate or fat result in an increase in the serum phenylalanine instead of the decrease described The high morning values may be reduced by giving a meal of Albumaid® late in the evening We presume that the phenylalanine free protein hydrolysate induces protein anabolism in the course of the day by supplying the organism with the essential amino acids In the child with phenylketonuria the phenylalanine necessary for protein anabolism is taken from the phenylalanine excess in the watery phase in the organism The increase in the phenylalanine concentration from midnight until the forenoon hours must be presumed to express tissue protein catabolism during these hours of the day On the basis of intravenous phenylalanine tolerance tests it was found possible to calculate the phenylalanine capacity in two children with phenylketonuria Thereafter it proved possible to calculate the net increase in phenylalanine

hours Forty eight of the children were followed up at least five years after the first admission No clinical evidence of malabsorption could be found in 79 • and *d* xylose tolerance tests gave normal results in the majority of cases In 12 of the cases both *d* xylose tolerance tests and renewed fat tolerance tests were undertaken Out of nine children with normal *d* xylose tests, four had steatorrhoea It is concluded that the *d* xylose tolerance test is invaluable as a screening test for steatorrhoea

*Discussion*

*J Vesterdal* How was the material selected? Many very slight cases of dyspepsia appear to have been investigated

*C J Ingomar* All cases of dyspepsia including the slight cases admitted during a definite period were investigated

*P W Bræstrup* Were the patients treated between the first treatment and the follow up examination?

*C J Ingomar* Patients with gluten intolerance or presumed gluten intolerance were treated with a glutenfree diet

*E Thamdrup* Was the fat determined diet administered for a period prior to collection of the stools for examination?

*C J Ingomar* No

## Meeting Oct 14 1968

Margaret Jones (Los Angeles) *Diagnosis and management of the infant with delayed motor development*

## Meeting Nov 13 1968

Erik Wamberg *The background and aims of the Kennedy Institute*

The John F Kennedy memorial collection in 1964 resulted in over 1 million Danish crowns (£50 000) The committee decided to employ this sum for the inauguration of a home for treatment of and research into the disease phenylketonuria Experience abroad had revealed that difficulties must be anticipated in carrying out the strict dietary regime necessary in the childrens own homes and consequently it seemed reasonable to establish a special home for treatment of this group of children for whom prolonged periods of admission to hospitals or research institutes is not suitable

The Institute was erected on a site which had been purchased by the Danish National Service for the Mentally Retarded in 1961 near the State Mental Hospital in Glostrup The buildings are built round an open courtyard and have accomodation for 15 infants and/or toddlers In addition to a large diet kitchen designed for demonstration of preparation of diets there are a couple of guest rooms for visiting relatives a large common room and accommodation for secretaries and laboratories

The tasks of the Institute which are mentioned briefly include not only dietary treatment of the inpatients and control examination of children living at home but also elaboration of new diets and advisory diagnostic, dietetic and genetic functions for parents medical collagues and institutions Further research activity is undertaken which in addition to investigations in connection with phenylketonuria also includes a rapidly increasing number of chromosome investigations undertaken in the chromosome laboratory established in the Institute

Erik Wamberg *The principles and experience in dietary treatment of phenylketonuria*

By way of introduction the principles of dietary treatment which have become established in the various centres of treatment all over the world on the basis of the experience gained in the past 15 years are mentioned In various centres in Germany Britain U S A and Canada, different schools of thought have gradually developed and their points of view can diverge considerably In the Kennedy Institute during the past year in which treatment has been undertaken we have arranged this according to the experience gained by Berry & Sutherland from Cincinnati As diagnostic criteria, serum phenylalanine of 15 mg with rapidly increasing tendency together with a low serum tyrosine and possibly demonstration of ketone bodies in the urine in newly born infants are considered to be adequate indications for the commencement of dietetic treatment The differential diagnostic deliberations involved in the various forms of hyperphenylalaninaemia are mentioned and the relevant blood and urine investigations are described

If doubt arises as to the justification of dietary treatment of an infant in the first months of life the diet may be replaced by an ordinary milk mixture for some days with simultaneous observation of the serum phenylalanine values Should these increase markedly the diet can be resumed

Deliberations concerning the choice of the dietary preparation (low phenylalanine or phenylalanine free protein hydrolysate) and the reasons for employing the English preparation Albumaid[R] are reviewed after the methods of determining the daily requirement of phenylalanine and the arrangement of the diet are outlined

In the Kennedy Institute we attempt to maintain a therapeutic level of serum phenylalanine of between 3 and 7 mg as proposed by Berry & Sutherland estimated in fasting blood samles While observing the diurnal variations in the phenylalanine values in the treated children we have demonstrated that the fasting values are highest and are followed by a steady decrease in the course of the day corresponding to the anabolism which occurs and which is mentioned in the preceding contribution

Our experience hitherto and that of many other authors appears to indicate that adjustment to too low a therapeutic level involves a considerable risk of overtreatment with subsequent deficiency symptoms which manifest themselves in failure to thrive possibly loss of weight or failure to gain weight combined with apathy anorexia anaemia exanthemata loss of hair hypoglycaemia and occasionally seizures

Finally the course of treatment in individual case is described and illustrated by the course of the serum phenylalanine values

*Discussion*

*O Steinicke* How have the first treated cases developed mentally? How early must treatment be commenced to achieve results?

*E Wamberg* Out patients have not yet been tested psychologically Treatment should be commenced prior to the third month of life and be continued for five to six years

*E Thamdrup* How many patients have been treated in the Kennedy Institute?

*E Wamberg* We have seven inpatients at present and have treated a total of 15

*J Vesterdal* Is the incidence of 1 per 10 000–20 000 correct?

*E Wamberg* We do not see the untreated backward children or the patients with normal intelligence According to the health visitors reports the incidence is 1 per 27 000 *births* and according to the results of the Guthrie test 1 per 10 000 births Probably the incidence is 1 per 20 000 births i e 4–5 cases annually in Denmark

resulting from tissue protein catabolism to 100 mg for a child of about 15 kg This corresponds to a protein catabolism of 2 g Approximately just as much again is lost by excretion of phenylalanine and phenylalanine products in the urine sweat, hair nails and skin The net protein catabolism may thus be calculated to be 4 g per night

It is mentioned that protein metabolism at night may be a normal or an abnormal phenomenon because tyrosine is an essential amino acid for children with phenylketonuria Administration of tyrosine at night does not counteract protein catabolism

When a phenylalanine free protein hydrolysate is available for dietetic treatment, it appears from our most recent investigations that it is possible to adjust the phenylalanine level to a certain extent by balancing the quantity of phenylalanine containing diet (natural protein) against the quantity of essential amino acids without phenylalanine (Albumaid®) Increased serum phenylalanine may be lowered by inducing increased protein anabolism with a phenylalanine free protein hydrolysate If this solution is chosen, rather than a reduction in the administration of normal protein normal growth becomes possible

Erling Olesen *The amino acid pattern in a phenylalanine free protein hydrolysate (Albumaid® Scientific Hospital Supply Liverpool)*

In dietetic treatment of children with phenyl ketonuria treated in the Kennedy Institute, a phenylalanine free protein hydrolysate (Albumaid®) has been employed to supplement the strict low protein diet

In the dosage usually employed the protein hydrolysate contributes approximately $^{3}/_{4}$ of the total supply of amino acids On determination of the content of individual amino acids in the preparation it was found that the quantity of methionin was considerably lower than that stated by the manufacturers The distribution of the essential amino acids administered via diet + protein hydrolysate revealed as compared with the requirements in infants given by Holt & Snyderman and with the amino acid pattern in a typical normal diet, considerable divergence with low values for methionin and isoleucin

A corresponding impression that the protein hydrolysate has a relatively low content of methionin and isoleucin and to a lesser extent also leucin is obtained by comparing the content of essential amino acids in Albumaid® with the content in whole egg protein The latter is recommended by the FAO WHO expert committee as a pattern for reference as the distribution of the essential amino acids in whole egg is presumed to be near the optimal both for growing and for adult individuals

For use in further investigations the preparation has therefore been supplemented with *l* methionin *l* isoleucin and *l* leucin in such quantities that the distribution of essential amino acids corresponds to a greater extent to that in whole egg protein (which does not differ to any great extent from the pattern in breast milk or cow's milk) Future investigations will reveal whether this supplement of amino acids which must be presumed theoretically to imply an improvement of the phenylalanine free protein hydrolysate, will result in clinically demonstrable advantages e g such as increased phenylalanine tolerance better thriving and the possibility of reducing the dosage

*Discussion*

*J Vesterdal* Why was hens egg employed instead of breast milk as the normal reference? Renal damage resulting from too high dietary protein is never observed but a diuretic effect occurs as a result of increase in blood urea

*E Olesen* Hens egg is given as a reference by an expert group from FAO which was not concerned solely with infants

patients have thus a 2.5 per cent risk of having pancreatic fibrosis in addition, i.e. 1 per 40 patients.

During the next six months all of the patients registered under the Service for the Care of the Mentally Subnormal will be investigated in order to elucidate the extent and clinical features of the cri du chat syndrome.

*Discussion*

*A. Dupont*: In order to illustrate the incidence of the syndrome sought by Dr. Niebuhr, I can give the following information.

In our chromosome laboratory we have investigated 583 mentally retarded patients and revealed 52 with chromosome anomalies. Two out of these 52 chromosome anomalies were cases of the cri du chat syndrome.

*The first patient*, a male aged 20 years, was found when Dr. Petrea Jacobsen investigated 100 patients with the combination of mental retardation and congenital malformations. One hundred mentally retarded patients without congenital malformations were investigated as controls.

This patient is not particularly typical and would scarcely have been found on clinical evidence alone. His only malformations are slight malformation of the external ears (poorly developed lobulus), abnormal dental development, transverse palmar creases in both hands, gap between the first and second toes on both feet and slight neurological symptoms.

The voice is not characteristic. The chromosomes in the parents did not show any abnormality. Dr. Margareta Mikkelsen kindly undertook autoradiography in both of our patients and demonstrated that deletion of the short arms of chromosome no. 5 was concerned.

*The second patient*, a girl aged three years, was found during routine investigations of all newly registered cases in the Centre. She is much more typical and shows a series of the cardinal symptoms and has, in addition, a little skin tag anterior to the right ear corresponding entirely to the first case described (Dyggve & Mikkelsen).

Slides demonstrating the chromosomes and photographs of the two patients were shown.

Meeting Dec. 9, 1968

*K. Mauritzen*: *An Eisenhower Fellowship. Report of a six month visit to U.S.A.*

*P. Paerregaard*

*A Dupont* I have seen two untreated feeble minded patients

*N Hobolth* We have seen two new cases in Kolding from the age of a few months A similar plan for screening for myxoedema in newly born infants should be elaborated and enforced

Erik Nieburh *The Cri du Chat syndrome*

Since the first description of this condition by Lejeune and his co workers in 1963 112 similar cases have been described By comparing the clinical symptoms in these patients the following main symptoms may be emphasized an abnormal cry (a cat like scream) microcephaly hypertelorism epicanthus antimongoloid orbital fissures, low set ears, micrognathia transverse palmar creases mental retardation (imbecile or idiot level) and failure to thrive

In children under the age of two years all of these symptoms are present in more than 80 % of the cases but the clinical picture becomes progressively less distinct so that the diagnosis is difficult to establish in adults

The ratio ♀/♂ = 2/1 the average birth weight is 2800 g and the average paternal age is 29 years and maternal age 27 years The incidence and prevalence of the disease are unknown

By means of screening children under the age of 15 years registered under the Danish National Service for the Care of the Mentally Retarded Centre I (Greater Copenhagen and Bornholm) nine patients with the disease were found A total of 13 cases are known in Denmark

Chromosome investigation revealed deletion (i e breakage of a chromosome with loss of a greater or lesser fraction) of the short arms of chromosome no 5 in more than 90 of the cases and, in the remainder a ring chromosome mosaic condition rare chromosome deviations or apparently normal findings are found

The extent of the deletion has apparently no influence upon the degree of the mental deficiency but in patients with mosaicism less pronounced mental retardation may perhaps be anticipated

By means of autoradiography (addition of tritium labelled thymidin to the cultures) chromosome pairs nos 4 and 5 can be differentiated Clinically there is no difficulty in differentiating between patients with deletions of chromosomes nos 5 and 4 as the latter group of patients are dominated by defects in the mid line colobomata clefts of the lip and palate congenital heart disease and malformations of the renal and genital tracts very similar to Pierre Robin s syndrome (the latter is associated with normal chromosome conditions)

Chromosome investigation should also be undertaken in the parents as in 15–20 percent of the cases one of them will similarly show a chromosome defect (translocation) This figure is very great e g as compared with the incidence of translocation mongols The theoretical distribution of children when one parent has a translocation is 1/4 cri du chat syndrome 1/4 with trisomy for the short arm (these children die in the first year of life and have severe mental defect) 1/4 are clinically normal but carriers and 1/4 are completely normal

No known blood type or enzyme system has been found to be localised to the short arm of chromosome no 5 Smith *et al* (Lancet *II* 309 1968) however consider that the gene for pancreatic fibrosis is possibly localised here as they found a patient with pancreatic fibrosis and with the cri du chat syndrome whose mother was heterozygotic and the father homozygotic normal The child has thus only one abnormal gene and the reason for manifestation of the condition is possibly that the normal allele gene has been lost in deletion The incidence of the gene for pancreatic fibrosis is stated to be 5 % in U S A and cri du chat

bellum anlage like e g vascular disturbances in this region

In an individual suffering from hemorrhagic diathesis the bleeding may occur early in embryonic life during organogenesis as well as later Such cerebral hemorrhages with localized hematomas widespread extravasation in the region of the rombic lip and bottom of the fourth ventricle or destructions of the cerebral vesicles have been induced experimentally in chick embryos exposed to increased incubation temperatures Work to establish connection between such early pathological changes and later development of malformations are in progress

There are reasons to assume that most of the human embryos with the inherited congenital thrombocytopenia die as a consequence of bleeding Therefore great interest and effort should be paid to find the frequency of abortions in women transmitting this disorder to their offspring

*H M Hoyeraal* The mother has had one abortion and she has two healthy children We suggested as a possibility a thrombocytecontrolling centre in the brain an that the thrombocytopenia may be secondary to malformations of the central nervous system In mammals with short gestational periods thrombocyte production starts early Although megacaryocytes are seen early in the human fetus thrombocytes are hard to detect until the second half of gestation Thrombocyte regulation studies in rats revealed a feed back mechanism To us it seems unlikely that the same type of brain malformations in two siblings results from hemorrhage

Letten F Saugstad *EEG findings in children with perinatal injuries*

*Thor Oistein Endsjo*

# PROCEEDINGS OF PAEDIATRIC SOCIETIES

## NORWEGIAN PAEDIATRIC SOCIETY

### Meeting Oct 18 1968

Margareth H Jones (*University of California Los Angeles*) *Diagnosis and management of the infant with delayed motor development*

H M Høyeraal & P J Moe *Hypoplastic thrombocytopenia and central nervous system malformations in two brothers*

So far 32 cases of primary hypoplastic thrombocytopenia associated with other anomalies have been published The most common anomalies are Bilateral absence of radii (24 cases) other skeletal defects (16 cases) cardiac defects (7 cases) genito urinary tract anomalies (4 cases) and congenital spherocytic anemia (2 cases) Two brothers with thrombocytopenia malformations of the central nervous system and a non sperocytic hemolytic anemia are presented Both had low birth weight for maturity 2200 and 2400 g and bruised readily from the age of 9 and 5 months respectively Psychomotor development was severely retarded They were also markedly retarded in growth both were microcephalic with peculiar features and both had signs of cerebellar ataxia and spasticity particularly in the lower limbs The first child died in 1960 following a head injury Autopsy revealed a subdural hematoma and a small brain, 610 g, with a very small cerebellum weighing only 1 4 per cent of the whole brain Pneumoencephalogram in the surviving brother showed marked ventricular dilation with cortical atrophy

Abundant megacaryocytes were reported in the bone marrow in Case 1, while a persistent reduction in the number of megacaryocytes has been found in Case 2 Survival studies in Case 2 showed decreased life span of the patient's erythrocytes in normal blood normal survival of normal thrombocytes in the patient's blood

In Case 2 the toxoplasmosis hemagglutination test was positive at the age of 3 ½ years but the dye test was negative Clinical evidence of toxoplasmosis was not found in either Case 2 has received numerous blood and platelet transfusions Steroids have been of little benefit Splenectomy resulted in a transient rise in thrombocyte level

Family studies including blood analyses blood grouping immunoelectrophoresis and Hp Gc and Gm typing have shown no definite abnormalities but 5 of 26 cases had IgG below 8 mg per ml To our knowledge these are the first two cases of congenital hypoplastic thrombocytopenia associated with hypoplasia of the cerebrum and cerebellum and a non spherocytic hemolytic anemia The relationship of this syndrome to other cases of congenital hypoplastic thrombocytopenia and its possible pathogenesis was discussed

*Discussion*

*N Ohre Nilsen* The association between congenital thrombocytopenia and malformations has considerable teratological interest The abnormalities of cerebellum described in the paper presented can from an embryo pathological point of view possibly be considered due to a nonspecific general injury involving the cere-

Children's Hospital University of Helsinki In eight no thyroid cells were seen in aspirate A specimen was obtained in three hypothyroid four hyperthyroid and seven euthyroid patients In the first group there was one patient with a probable coupling defect highly active epithelial cells with cystic changes were seen In a child with the non butanol extractable iodine syndrome cells extremely rich of coarse granulation but without vacuolation as usually seen in hyperactivity of the epithelium were observed

Of the hyperthyroid goitres three gave a hyperactive and one a normoactive picture

Of the euthyroid goitres one was nodular The cytological picture was very polymorphous but the nucleoles were small which is inconsistent with malignancy Biopsy of a surgical specimen from the same thyroid revealed papillomatous adenoma

In six cases lymphomatous thyroiditis was suspected on the basis of clinical and laboratory investigation The cytological examination confirmed the diagnosis

Although experience with the method still is limited the value of the method was stressed and its use in the examination of all goitrous patients was advocated

Antti Korvikko *Effects of metabolic acidosis on the circulation of newborn lambs*

Since little information is available concerning cardiac function in newborns during asphyxia the present study of newborn lambs was undertaken with the aim to investigate the effects of metabolic acidosis hypoxia and hypercapnia on the circulation

The *metabolic acidosis* developed in these lambs spontaneously and following hypoxia Cardiac output decreased linearly with decreasing base excess (B E) Thus with B E of −10 mEq/l cardiac output had decreased by nearly 40 per cent from the normal This change was due to a decrease in stroke volume of about 35 per cent Also the central blood volume decreased by about 20 per cent When B E decreased to −20 mEq/l all of these parameters decreased further linearly

The *response to arterial hypoxia* in the lambs was influenced by the metabolic acidosis with a nearly normal B E (more than −5 mEq/l) the cardiac output increased by 31 to 35 per cent during hypoxia when the mean arterial O saturation was 67 per cent When B E was from −5 to −10 mEq/l the cardiac output did not increase significantly and with B E of less than −10 mEq/l it decreased slightly during arterial hypoxia This impaired response to arterial hypoxia was due to the decrease in stroke volume as the heart rate during hypoxia tended to increase by 5 to 10 per cent at all stages of metabolic acidosis

The metabolic acidosis did not seem to influence the cardiac *response to hypercapnia* The cardiac output increased always when the arterial $P_{CO_2}$ was increased by ventilating with 10 per cent CO The heart rate and stroke volume contributed equally to this increase of cardiac output

This study confirms the clinical observation that maintainance of sufficient oxygen transport and correction of metabolic acidosis improve the prognosis of infants suffering from RDS Earlier studies have confirmed that normal $P_{CO_2}$ and acid base balance improve the lung circulation and promote closing of the arterial duct According to the present study normal acid base conditions are important also for an efficient function of the heart of the newborn lamb

Antti Kumento *The serum binders of vitamin $B_{12}$ in newborn infants*

The binders of vitamin $B_{12}$ in the serum of newborn infants were studied using *in vitro* addition of radioactive cyanocobalamin Besides the previously described $B_{12}$ binders transcobalamin (TC) I and II a third binder was detected This fetal $B_{12}$ binder is eluted between TC I and TC II in DEAE-cellulose column chromatography Its molecular size is equal to that of TC I and its electrophoretic mobility is

# PROCEEDINGS OF PÆDIATRIC SOCIETIES

## FINNISH PÆDIATRIC SOCIETY

Meeting Oct 19 1968

### Rolf Nordman *Endemic goitre in Finland and the thyroid of newborn*

Endemic goitre has been a true nationwide disease in the greater part of Finland Its major cause is shortage of iodine in the daily diet

The thyroid of the newborn can be considered a good indicator of the nature of and changes in the endemic goitre situation In a goitre free area the thyroid may be so small at birth that it is not detectable by palpation It is about 1–2 g in weight according to autopsy series Typical of its histological structure are clearly evident follicles that contain colloid and an epithelium consisting of a single layer of cubical cells In the normal condition the thyroid of the newborn contains a relatively large amount of iodine

Particularly in investigating the effect of iodine prophylaxis the thyroid of the newborn is an expedient object of study allowing an early estimation of the result achieved

In studies of newborn thyroids in the 1930s and 1940s South Finland was found to be an area of moderately severe or slightly milder endemic goitre

In the present study no case of congenital goitre was found among 1600 babies born in the city of Hameenlinna The mean thyroid weight of the newborn was 1 4 g in Helsinki (123 thyroids) and 1 7 g i Joensuu (34 thyroids) In the total series the mean weight was 1 47 g

The percentages of epithelium and colloid in the thyroid of the newborn were calculated by means of an integration ocular In the total series the mean percentage of epithelium was 22 61 and the percentage of colloid 48 52

The total iodine content was determined in 70 thyroids of newborn infants The mean content was 351 $\mu$g or 206 $\mu$g/g

The serum protein bound iodine and cholesterol values in the newborn were also within the normal range The mean level of PBI was 6 34 $\mu$g per 100 ml and that of cholesterol 107 mg per 100 ml

Thus in the area studied the thyroid of the newborn is similar to that in areas free from endemic goitre in size weight histological structure and iodine content This favourable development can be attributed to iodine prophylaxis In the 1960s over 50 per cent of all the food salt sold in Finland has been iodized In the present series the mothers of the newborn had used iodized salt during pregnancy in nearly 100 per cent of the cases

### Tom Sederholm and Jorma Maenpaa *Aspiration biopsy in thyroid diseases*

The aspiration biopsy method introduced by Templa in 1948 and further developed by Soderstrom Franzen and Persson is safe and technically simple as compared to the standard procedure with the coarse Vim-Silverman needle Typical findings in different thyroid diseases were described and the possibilities and limitations of cytological examination and diagnosis with this method were discussed

During the last years 22 goitrous patients have been examined with this procedure at the

in the center of the cotyledon In the fourth cotyledon type the structure was obscure and individual arteries could not always be classified with certainty

When the occurrence of the above mentioned cotyledon types was studied in placentas filled in different ways it was found that well filled placentas always had plenty of type 1 cotyledons whereas in poorly filled placentas these cotyledons were missing or rare On the basis of this it seems possible that disagreeing opinions on the structure of the vasculature on the fetal side of the placenta are due to different techniques employed

Aro Relander *Intravenous galactose tolerance test in normal children*

The galactose tolerance test is generally used for the evaluation of liver function in adults

The lack of normal values for children has prevented the adoption of the galactose tolerance test for children

T ½ values were obtained with the galactose tolerance test following the method of B Tengstrom (*Scand J Clin Lab Invest 18* Suppl 92 131 1966) in 98 normal children To exclude pathological cases liver function was tested by determining serum bilirubin and GPT and by performing the thymol turbidity test A highly significant positive association was shown between age and T ½ values (r = 0 66) In the age group 0–5 years the upper limit of normal is 10 min in the age group 5–10 years 12 min and in the age group 10–15 years 15 min

Seppo Simila *Hyperprolinaemia type II* (To be published in *Ann Paed Fenn*

Matti Dahl & Pentti Rantanen *Severe prolonged hypothermy caused by Phenylbutazone in a child with Hodgkin s disease*

An 8 year-old boy with Hodgkin s disease had been treated for four years with roentgen therapy Degranol–Chinoin² (mannomustine) Natulan² (procarbazine cloride) and corticosteroids Because of acquired hemolytic anaemia associated with autoantibodies splenectomy had been done The disease then became resistant to roentgen therapy and the patient s condition deteriorated with a fever of 40 C Aminophenazone and salicylates had no effect on the fever At this stage an i m injection of 15 mg/kg phenylbutazone was given It caused hypothermy lasting for two days The injection was repeated later with the same result The rectal temperature fell at the first time to 32 5 C and at the second time to 33 5 C Six months previously the patient had been given a similar dose of phenylbutazone several times and his temperature had at that time gone down to the level of 36–37 C for about one day During the hypothermy periods the patient sweated strongly he did not feel chilled he liked to be without blankets and was a little tired, but in spite of this he watched TV During the hypothermy periods the leucocyte count was 5 800–10 700 with 7 0–3 0 per cent lymphocytes bilirubin 2 1–2 4 mg per *100 ml* and GOT 60–37 mIU/ml Later on the patient was given 5 mg/kg phenylbutazone in an i m injection but the smaller dose did not seem to have any noticeable effect on the temperature That excluded the possibility of an allergic reaction

The patient died about one month after the last hypothermic reaction At autopsy his liver was found to be infiltrated with lymphogranulomatous metastases

Hypothermic reaction is a less wellknown side effect caused by phenylbutazone It has been reported earlier in patients with Hodgkin s disease typhoid fever and brucellosis This complication may be associated with liver involvement or possibly with lymphopenia lymphopenia during the hypothermic reaction was a feature common to the different patients reported

Aarne Vartia & Ilkka Valimaki *The effect of some chronic cardiac arrhythmias on the physical working capacity of children*

Cardiac output is considered the most important factor limiting physical working capacity

between TC I and TC II The fetal $B_{12}$ binder does not contain endogenous $B_{12}$ The vitamin $B_{12}$ bound to it is not transferred into HeLa cells in *in vitro* incubation The function of this $B_{12}$ binding substance is unknown

Yrjo Partanen & Kristi Heinonen *Screening for neonatal hypoglycaemia*

Screening for neonatal hypoglycaemia was started a year ago at the Central Hospital in Mikkeli

All neonates in the maternity unit had Dextrostix® test at the age of 2–3 hours and again at 6–8 hours performed by the attending nurse In infants < 3000 g the test was further repeated twice during the second day In the infants transferred to the neonatal observation unit the test was done at 6 hour intervals during the first two days In addition, the test was obtained *immediately whenever symptoms suggestive* of hypoglycaemia occurred

Dextrostix® under 40 mg per 100 ml or 40 mg per 100 ml prompted a blood glucose determination with the aldosaccharide specific ortho toluidine method (Hultman Hyvärinen Nikkila)

Treatment (glucose i v and cortisol) was begun when two glucose determinations were reported below 30 mg per 100 ml in full term infants and below 20 mg in prematures or low birth weight infants Breast milk feeding was started as soon as the patients general condition permitted (mostly at 6 hours of age) The infants thus treated had Dextrostix® done every 6 hours and glucose determination three times during 24 hours until the second day after stopping the treatment

From Nov 15 1967 till Sept 10 1968 1000 live infants were born at the hospital As a result of the screening a monthly average of eight infants had blood glucose determinations Altogether four of these required treatment for hypoglycaemia Five other infants were transferred to the neonatal observation unit with a suspicion of hypoglycaemia which could not be confirmed

In the neonatal observation unit a total of 109 infants were treated Of them 15 had Dextrostix® under 40 mg per 100 ml The corresponding glucose determination showed hypoglycaemia in six instances and normoglycaemia in nine instances Fifteen infants had Dextrostix® 40 mg per 100 ml The corresponding glucose concentrations were all ≥ 40 mg per 100 ml The rest of the patients in the neonatal observation unit (70 infants) had Dextrostix® over 40 mg per 100 ml In 10 of them simultaneous blood glucose determination was performed for comparison the results were all over 40 mg per 100 ml

The commonest symptoms were unspecific decreased muscular tone, twitchings and respiratory disturbances

Kari Raivio *Blood glucose of the human fetus prior to and during labor*

(Published in *Acta Paediat Scand* 57 512 1968)

Kai Krohn Biorn Ivemark & Kalle Salo *The fetal arterial pattern of normal human placenta*

The microangiographic technique was employed in the investigation of 36 human placentas The main purpose was to classify the structure of the vasculature on the fetal side of the placenta since opinions on this matter are partly in disagreement Two types of intracotyledonar arteries existed in the fetal cotyledons of the placenta The arteries of type A were thin straight and their branches ran inside the villus stem Without exception these arteries extended from chorion to decidua The arteries of type B were thicker and twisted and their branches ran in their own separate villus stem often turning perpendicular or opposite to the main artery Depending on the presence of the above mentioned artery types in the cotyledons the latter could be grouped in four types, so that in type 1 there were only A type arteries in type 2 only B type arteries In type 3 both A and B type arteries occurred and the B type arteries were located in this type always

in the center of the cotyledon In the fourth cotyledon type the structure was obscure and individual arteries could not always be classified with certainty

When the occurrence of the above mentioned cotyledon types was studied in placentas filled in different ways it was found that well filled placentas always had plenty of type 1 cotyledons whereas in poorly filled placentas these cotyledons were missing or rare On the basis of this it seems possible that disagreeing opinions on the structure of the vasculature on the fetal side of the placenta are due to different techniques employed

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Aarne Varta & Ilkka Valimaki *The effect of some chronic cardiac arrhytmias on the physical working capacity of children*

Cardiac output is considered the most important factor limiting physical working capacity

Some factors decreasing cardiac output are a heart rate far from optimal irregularities lack of atrioventricular synchronization and inability to adapt to exercise

The physical working capacities ($PWC_{170}$) of seven children with congenital A V block one girl with regularly appearing ventricular ectopic beats and one boy with constant auricular flutter (and a large ASD) were estimated on a bicycle ergometer according to Wahlund (*Acta Med Scand* Suppl 1948) and Holmgren (*Acta Med Scand* 1959) Their ages ranged from 6 to 14 years The ECG was continuously recorded during the exercise and one hour thereafter with an ECG tape recorder (Electrocardiocorder by Holter Avionics)

In the A V block patients the ventricular rate was 41 to 55 per minute at rest and it rose during exertion practically in proportion to the auricular rate Ventricular extrasystoles were detected in the recordings during the highest load in two patients

There was a negative correlation between PWC and the ventricular rate at rest and on the other hand between PWC and the corresponding ventricular rate

PWC was within normal limits (when compared with the body surface area) in all cases except one The hemodynamic adaptation seemed to have compensated satisfactorily the disadvantages due to the block

The girl with ventricular ectopic beats occurring in 1 2 relation to the normal beats showed a normal $PWC_{170}$ The arrhythmia disappeared at the work load of 300 kpm/min and was also absent during normal walking

The boy with atrial flutter and ASD had no signs of congestive heart failure but the exercise tolerance was subjectively reduced The exercise had no effect on the auricular rate and only a minimal acceleration was found in the ventricular frequency in contrast to the congenital A V block cases Thus the PWC could not be estimated exactly but it was apparently reduced as the boy was near exhaustion at a subnormal load It is difficult to conclude whether the reduction was due to the ASD or the arrhythmia

Jussi Vilska *Present stage of the international study of the nephrotic syndrome*

A preliminary report on the results of the international study of the nephrotic syndrome in children directed by Dr Henry L Barnett in New York was presented

Meeting Nov 23 1968

Ole Wasz Hockert *Optimal therapeutic schemes of tuberculous meningitis in childhood*

Ossi Pettay *Intrauterine viral infections*

Alf Backman *A skin test to detect active tuberculosis in BCG vaccinated patients*

Eero Vapaavuori 1 *Recording of intratracheal pressure during artificial ventilation (IPPR) in respiratory insufficiency of the newborn* 2 *Results of respirator treatment (IPPR) in various types of respiratory insufficiency of the newborn* 3 *Intensive care of small premature infants (850–1250 g) Treatment and results*

These papers were also read at the XII International Congress of Pediatrics in Mexico on Dec 1–7 1968 and abstracts of the papers will be published in the Congress Proceedings

*E J Hallgren*

# BOOK REVIEWS

. Meyler & H M Peck (eds) *Drug Induced Diseases* Vol 3 340 pp Excerpta Medica Foundation Amsterdam 1968 US $70 00

Information to the medical profession about new side effects of drugs should be both rapid and practically useful Thus both early warnings published in the medical journals or issued by Adverse Reaction Committees and regular reviews of the literature on side effects are needed This book which now appears in its third volume belongs to the latter type of information It contains papers written by invited authors who are reviewing our present knowledge about the mechanisms and clinical implication of the side effects produced by specific drugs It is not a complete survey but emphasis has been laid on common drugs that are widely used

The book reflects the now well known difficulties involved in applying statistical method in this area of clinical pharmacology For instance in the careful review by Tarck in which the possible connection between oral contraceptives and thrombosis is discussed it is pointed out that there are still wide divergencies of opinion in the literature about the real incidence of this complication in women taking the pill Another example is a survey of the literature on the occurrence of the Stevens–Johnson syndrome following long acting sulfonamides where it is concluded that there is yet no proof that this reaction is more common in children than in adults

Anti infective compounds is another widely used group of drugs with a variety of possible side effects This is reflected in several articles The recently described pulmonary reaction to nitrofurantoin is a good example of side effects that have probably been underestimated in their incidence because the physicians have not been aware of their existence

The article by Toaff & Ravid should be especially interesting for the pediatricians It is an excellent review of the tooth discoloration due to tetracyclines including discussion of the mechanism behind the deposition of these drugs in bones and teeth A valuable bibliography is included as in several of the other articles

Other articles deal with aseptic bone necrosis and hematolog cal consequences of corticosteroid therapy malabsorption due to prolonged administration of neomycin hyperglycemia from thiazide diuretics etc A few chapters on more general themes are also included like Drug dependence Placebo induced side-effects and Drug hazards in the elderly"

Books of this kind might have a short life time but should nevertheless be available in the clinical library

*Lars O Barrus*

H F R Prechtl & D J Beintema *Die neurologische Untersuchung des reifen Neugeborenen* 81 pp illus George Thieme Verlag Stuttgart 1968 DM 14 80

In 1964 Prechtl and Beintema wrote a manual of the neurological examination of the fullterm newborn infant This book published as Little Club Clinics in Developmental Medicine No 12 was very gratefully received and became rapidly known all over the world as a most objective and detailed description of how to examine and how to evaluate the different findings in this peculiar age group The English guide for examination has now been translated and extended by Prechtl into a new German edition Valuable minor modifications have been made A useful scheme to summarize and estimate a routine examination has been added in a special part at the end of the book A practical neurological examination for quick screening will also be found in this part of the book —This new German edition is highly recommended particularly as its comfortable size makes it very suitable for every-day use in the pocket of the doctor s white coat

*Bengt Hagberg*

M Nennan J Montant & J Sapelier *Epanchements Sous Duraux Chroniques du Nourrisson* 207 pp illus L'Expansion Scientifique Paris 1968 56 30 F

This monograph is a detailed description of the symptoms the diagnosis and the treatment of 87 cases of subdural hygromas among infants and children at the Pediatric Department in Nantes 1955 to 1967 Besides trauma at delivery several other etiologic factors have been found among these cases e g purulent meningitis severe malnutrition and severe gastroenteritis with disturbances of the physiologic osmolality of body fluids as well as the battered child syndrome The authors have included neuro radiological methods such as air-encephalogram and carotis angiography in their diagnostic procedures where as simpler methods such as eco-encephalograms and skull transillumination have only been used in a few situations In the first years of this period the treatment comprises only repeated subdural taps but later on this procedure was abandoned and replaced by more or less extensive neurosurgical operations

A follow up of the cases is unfortunately not presented and therefore the long term results cannot be evaluated This extensive monograph is accomplished with a reference list comprising 429 references Consequently it is most useful as a reference of literature about subdural hygromas in infancy and child

hood although no considerable novelties are contributed by the monograph itself

*Nils H. Svenningsen*

C. E. Alken, V. W. Dix, W. E. Goodwin, H. M. Weyrauch & E. Wildbolz (eds.) *Encyclopedia of Urology. Vol. VII/1. Malformations.* 479 pp. 348 Figs. Springer Verlag, Berlin, Heidelberg, New York 1968. US $49.00. DM 196.—

The comprehensive text book is written by twelve well known American experts. It gives a detailed presentation of the urogenital malformations. Some of the opinions in the book are however open to discussion. As a whole it gives an excellent presentation of the subjects. The typography is very attractive and the text book is higly recommended to surgeons and urologists.

*N. O. Ericsson*

Preben Geertinger: *Sudden Death in Infancy.* 107 pp. illus. Charles C. Thomas Publ. Springfield, Ill. 1968. US $6.75.

Sudden unexpected death (SUD) in infancy occurs particularly during the first year of life with a maximum between the age of two and four months. Boys are particularly affected and there is a peculiar seasonal variation with a predominance of SUD in winter. In the typical case the autopsy findings are essentially negative save petechiae on the chest organs.

Among the more and less valid theories which have been presented to explain these "cot deaths" are the ancient concept of "status thymicolymphaticus" (buried after the discovery of this condition in about one half of the young soldiers who were killed and autopsied during World War I) and hypersensitivity to cow milk. Some authors reporting peribronchiolar mononuclear infiltration and infectious hyperplasia in lymphnodes and spleen have attributed SUD to acute bacterial or viral infection.

The present book of Preben Geertinger is based on an analysis of 164 consecutive cases of SUD autopsied at the University of Forensic Medicine, Copenhagen, during a six year period (1957–1963). In 121 of these cases the traditional pathology failed to reveal any acceptable cause of death. The author now presents histological and biochemical evidence (the latter the result of postmortem examination of blood, bone and cerebrospinal fluid) that the SUD syndrome is caused by underdevelopment of the parathyroids, "the most difficult organs in the body to find" (p. 59) with disturbance of calcium metabolism and that these infants die from a sudden hypocalcaemic catastrophe. It is further suggested that the condition is acquired in utero and due to maternal abnormalities in calcium metabolism. The histological findings in particular are convincing in showing that a high percentage of the victims of SUD display abnormal parathyroid glands with inaction or fusion of parathyroid and thymic tissue. However, this is illustrated with an unnecessarily great number of microphotographs, some of which are of poor quality.

The hypothesis of parathyroid dysfunction acquired in utero is tested experimentally on a series of rats. It is shown that the offspring of mothers which during gestation were treated with a "phosphate robber" (aluminium hydroxide–magnesium carbonate gel) show a high incidence of incompletely developed parathyroids with fusion of parathyroid and thymic tissue. These experimental studies strongly support the theory of the author, but it might be questioned who no studies on the calcium metabolism of the mothers of SUD infants are reported or at least recommended.

The author sometimes seems to be affected by considerable bias, e.g. in the analysis of the histological findings in the kidneys, where changes were "particularly sought for" and an amazingly high incidence of "glomerular fibrosis" is reported. No reference is made to cerebral calcification, a notorious feature of hypoparathyroidism. Some of the tables give incomplete information and statistical analysis of significance levels is lacking.

Without solving all the problems of SUD this book is a vigorous challenge to former theories in this field. Biochemical, endocrinological and embryological research is called for to test the views presented in this small but stimulating volume.

*Bengt Robertson*

E. Rossi & E. Stoll (eds.) *Cystic fibrosis.* Proc. 4th Internat. Conf. on Cystic Fibrosis of the Pancreas 1966. Part II. 320 pp. illus. S. Karger, Basel, New York 1968. DM 75.—

This book contains several excellent review articles written by prominent scientists in the field of glycoprotein biochemistry. A number of articles deal with the current problems in this area, i.e. the polymorphism and microheterogeneity of glycoproteins and mucopolysaccharides (Schmid, Cunningham & Rodén). Studies on the structure of the linkage between carbohydrate and protein are also reviewed (Neuberger, Gottschalk & Rodén).

Another area which is elegantly expounded is the mechanism of biosynthesis of glycoproteins and related substances (Winzler & Roseman).

Since chemistry and biosynthesis occupy the major part of the symposium the important problem of the subcellular localization of the enzymes involved in biosynthesis as well as the intracellular processing of glycoproteins has received less attention. The same is true for the potential biological roles of these substances. The latter deficiency is most likely due to the relative lack of knowledge concerning the biological function of these macromolecules. It is obvious that the present symposium ought to stimulate both the novice and the expert in the field. It should b

added that the discussion also has a stimulating effect

Finally it should be pointed out that Dr Saul Kaman has performed a praiseworthy editing con tribution which has had a most beneficial effect on the big time between the date of the symposium and the publishing of the book This also adds to the value of this volume

*Lars Åke Fransson*

H Opitz & F Schmid (eds) *Handbuch der Kinderheilkunde* Vol 9 *Pädiatrische Grenzgebiete Augen Ohren Zähne Haut* (ed H Mai) 968 pp Springer Verlag Berlin Heidelberg & New York 1968 DM 315—

The series of *Handbuch der Kinderheilkunde* has earned itself a name of being one of the most comprehensive sources of knowledge within its special field This volume which is the 9th deals with diseases of the eye the ears the teeth and the skin

It is not possible to give a complete review of this book of 968 pages but certain features are evident after having used it as a handbook for a short time and gone through various sections

As a pediatrician one especially wants a handbook to give complete up-to-date knowledge about a certain disease This is not only in order to be able to make a correct diagnosis and to give the right treatment but it is of equal importance to know what to tell the parents who require information about the prognosis However in none of these aspects is the book satisfactory First of all it should be easy to orientate oneself in a handbook like this and in this respect the editing of the present book leaves something to be desired In many instances the same diseases have been described in different places by different authors and sometimes even under different names For example the index gives different pages for Robin's syndrom and for Pierre Robin's syndrom (which is the same) and neither the index nor the text gives any cross references here The same applies for Sutton's naevus and leucoderma acquisitum centrifugum The reason for this seems to be that the same disease often has been described by different authors (naevus flammeus by at least three different authors) without cross references in the text and different treatment has been recommended by the authors (one advises irradiation others do not)

Another example is that dysostosis mandibulo-facialis Franceschetti's syndrome Franceschetti–Zwahlen's syndrome and Treacher–Collin's syndrome are given separately in the index with different page references and the text does not explain that this is all the same condition

Another example of lack of balance in the presentation is that the description of zoster covers seven pages in spite of the fact that this condition is extremely rare in children whereas subjects of great pediatric importance such as verrucae plantares oxyuris vermicularis and retrolental fibroplasia have been discussed in only a few lines each and a disease like Letterer–Siwe's disease has not been described at all

References to the literature are given in each section and the list is usually long and often includes many old references which are important for the history of a given disease but since less than 10 per cent seem to refer to the English literature many of the original modern works have been omitted

Some of these blemishes could have been avoided by critical editing

This criticism does not change the fact that the book contains a wealth of information and plenty of splendid illustrations for which reason the book is very useful

*Bent Friis Hansen*

# ANNOUNCEMENTS

## THE 3RD EUROPEAN SYMPOSIUM ON EPILEPSY

This symposium is arranged by the International Bureau for Epilepsy The International League against Epilepsy and the Danish Epilepsy Association

The symposium will take place at Hotel Marienlyst Elsinore (near Copenhagen) Denmark on the 21st–23rd June 1970 The medical topics will be Etiological factors in the epilepsies (A) Genetic factors (B) Prenatal and perinatal factors

The social and psychological topics will be (A) Life insurance and invalide pension (B) The interaction between the epileptic child and teenager and the milieu The symposium will be activated by panel discussions and group discussions Further information and preliminary application DIS Congress Service 36 Skindergade DK 1159 Copenhagen K Denmark

## THE WORLD HEALTH ORGANIZATION REQUIRES SPECIALISTS IN MATERNAL AND CHILD HEALTH

Applications are invited for posts of Medical Officers in Maternal and Child Health Some vacancies exist and others are likely to occur in one of the WHO Regional Offices (New Delhi and Brazzaville etc) and in field programmes of assistance to Member Governments Applicants must be graduates of recognized medical schools and have postgraduate training in paediatrics and/or obstetrics Applicants should be experienced in health administration on country or city level In organization of maternal and child health services partly at least in developing countries in practical paediatric work in hospital and field work in maternal and child health in the teaching of medical students Experience in original research work preferred

An excellent knowledge of English or French with a good working knowledge of the other as a second language desirable

Persons who meet these requirements are invited to write immediately quoting as reference MCH Medical Officer Vacancies giving a summary curriculum vitae Those having the requisite preparation and experience will be requested to complete an application on the prescribed WHO form for active consideration

The Personnel Chief
World Health Organization
Avenue Appia
Geneva
Switzerland

*Acta Pædiat Scand 58* 561–566 1969

# PLACENTAL TRANSFUSION IN THE PREMATURE INFANT WITH OBSERVATION ON CLINICAL COURSE AND OUTCOME

A. C. YAO, J. LIND, R. TIISALA and K. MICHELSSON

*From the Midwifery Institute, Helsinki, Finland and Department of Paediatrics, Karolinska Institutet, Stockholm, Sweden*

This report describes the results of our investigation on the blood volume of the premature newborn infant following early and late clamping of the umbilical cord. Our effort is to find out whether placental transfusion occurs in the premature as in the full term and how it affects the clinical course of these infants.

## SUBJECTS AND METHOD

The subjects include 83 low birth weight infants born at the Midwifery Institute, Helsinki, Finland, as outlined in Table 1.

The ten "small for date" infants (group IV) had more mature physical features than their weights indicated. And they showed mild to moderate signs of soft tissue wasting. Their birth weights were below the 10th percentile for their gestational ages except in 3 (2 in the early-clamped and 1 in the late clamped group) who were below 25th percentile (12). All had a normal clinical course.

Early and late cord clamping was based on results of our previous study in the full term infant (24). From that study 3 early-clamped and 2 late clamped full term newborn infants of normal weight were included here for comparison (group I).

The umbilical cords were clamped early in 40 infants (39 less than 10 seconds and one at 15 seconds) late in 43 infants (34 at three minutes, seven at one minute, one at four minutes and one at five minutes). Birth was timed by stopwatch when the buttocks were delivered and cord clamping timed in reference to it. Onset of breathing and crying was also observed in relation to clamping. All mothers received intramuscular methergine injection immediately after birth of the infants. No anesthesia or sedatives were used. Although the time of cord clamping was carried out randomly there were more early clamped cases in the truly premature group with birth weight below 2001 g. Because of the higher incidence of birth asphyxia in this group requiring immediate resuscitation, early clamping was considered necessary.

Blood volume determinations were carried out within one hour of age as a rule but in 16 infants where delay was unavoidable the measurement was carried out within 1½ hours in 11 and within 2 hours in 5.

These 16 cases belonged to the following groups: group II, 3 in early, 2 in the late-clamped infants; group III, 4 in the early, 3 in the late-clamped infants; group IV, 3 in the early and 1 in the late clamped infants.

The procedure was performed in the treatment room on the delivery floors for the normal and larger premature infants. For the smaller premature infants and those who had difficulties at birth it was performed in the Isolette in the premature unit. ¹³¹I tagged human serum albumin dilution technique as in the Volemetron was employed. Dosage ranged from the umbilical vein immediately after cord. One drop of Lugol solution was given to each infant before the procedure and for two subsequent days.

The procedure used was essentially that described in previous communications (23, 25). Injection site was the scalp vein or the antecubital vein. The heparinized premix blood sample (2 ml) was obtained from the umbilical vein immediately after cord clamping. The 2 ml postmix blood sample was obtained 5 minutes after injection of the tracer from the femoral vein in most of the larger premature infants (in a few from the posterior venous sinus) and via an umbilical vein catheter (No. Fr 5–Fr 8 polyethylene feeding tube) inserted under aseptic condition, in the small premature infant and those with clinical signs of asphyxia. The umbilical stump was ligated or sutured and aseptically dressed in the usual manner after withdrawal of the polyethylene catheter. The radioactivity of the blood samples was then counted in the Volemetron, where blood volume was calculated. Venous hematocrit in triplicate was determined by the microcapillary method. The mean total blood volume was corrected for the difference in body hematocrit by the factor 0.87 (13).

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Table 1 *Data concerning the infants studied*

| Group and time of cord clamping | Birth weight range and (mean) in grams | Gestational age range and (mean) weeks | No of cases | Prenatal complications threatened abortion | Toxemia of pregnancy | Other causes |
|---|---|---|---|---|---|---|
| I Full term normal weight | | | | | | |
| (a) early-clamped | 2950–4700 (3446 9) | 37 5–43 (40 2) | 23 | 0 | 0 | 0 |
| (b) late-clamped | 3050–4540 (3531 4) | 37–42 (39 8) | 22 | 0 | 0 | 0 |
| II Truly premature 2001–2500 g | | | | | | |
| (a) early-clamped | 2100–2480 (2264 7) | 32–36 (34 5) | 17 | 1 | 2 | 2 |
| (b) late-clamped | 2020–2420 (2214 8) | 30–36 5 (35) | 27 | 1 | 5 | 2 |
| III Truly premature less than 2001 g | | | | | | |
| (a) early-clamped | 800–2000 (1694 1) | 26–36 (32) 9 cases < 32 | 17 | 3 | 2 | 3 |
| (b) late-clamped | 940 1970 (1665 1) | 29–35 5 (32 5) 7 cases < 32 | 12 | 2 | 1 | 1 |
| IV Full term less than 2500 g | | | | | | |
| (a) early-clamped | 1950 2430 (2216 6) | 38–40 (38 2) | 6 | 0 | 1 | 0 |
| (b) late-clamped | 1880–2430 (2267 5) | 37 5–40 (38 4) | 4 | 0 | 3 | 0 |

Includes ablatio placentae diabetes nutritional anemia and severe bronchial asthma

Idiopathic respiratory distress syndrome (IRDS) was diagnosed clinically based on findings of persistent tachypnea of over 60 per minute after the first hour expiratory grunting intercostal subcostal and sternal retractions cyanosis when breathing room air and characteristic chest X ray findings Hyaline membrane disease was found at autopsy of those who died Usher's therapeutic regimen was followed in the management of these infants (22)

## RESULTS

The results are presented in Table 2 and Figs 1 and 2 There was significantly higher blood volume, red cell volume and venous hematocrit in the late versus early clamped infants in all the groups studied, indicating that placental transfusion occurred when clamping of the umbilical cord was delayed The plasma volumes were similar between the early and the late clamped infants in the respective groups Among the late clamped infants the blood volume of the Group III b infants showed no significant difference from those of Groups I *b* II *b* and IV *b* although their red cell volume appeared significantly less There was no statistically significant correlation between the blood volumes red cell volumes and plasma volumes venous hematocrit and the parity duration of labor maternal age placental weight, cord length occurrence of the first breath or length of the infant

In the cases where the cords were clamped at 1 minute instead of 3 minutes, there was no significant difference in the blood and red cell volumes This was also true in the one case clamped at 4 minutes and in another at 5 minutes Although the number of cases were too few to draw any definite conclusion from the findings were not inconsistent with those of the normal full term infants studied by us where no significantly larger blood volume was demonstrated when the cords were clamped after 1 minute and the mothers received intravenous methergine during the third stage of labor (24) When the blood and red cell and

*Table 2 Mean blood red cell plasma volumes and venous hematocrit of 73 premature 10 low birth weight full term and 45 normal term infants*

CBV Corrected blood volume RCV = red cell volume PV = plasma volume M ± S.D = mean ± standard deviation

| Group and time of cord clamping | No of cases | CBV (ml)/kg M±S D | RCV (ml)/kg M±S D | PV (ml)/kg M±S D | Ven Hct (%) M±S D |
|---|---|---|---|---|---|
| I Full term normal weight | | | | | |
| (a) early-clamped | 23 | 71 3±8 2 | 31 3±4 3 | 39 6±4 8 | 50 2±2.9 |
| (b) late clamped | 22 | 89 5±12.2 | 47.5±6 1 | 42.6±7 1 | 60 7±3 8 |
| p-value | | <0 001 | <0 001 | — | <0 001 |
| II Truly premature 2001–2500 g | | | | | |
| (a) early-clamped | 17 | 74 6±11 4 | 32 3±4 9 | 42 3±9 3 | 50 1±6 5 |
| (b) late-clamped | 27 | 88 8±10 1 | 47 5±7 1 | 41 5±6 3 | 61 2±5 9 |
| p-value | | <0 001 | <0 001 | — | <0 001 |
| III Truly premature less than 2001 g | | | | | |
| (a) early-clamped | 17 | 68 8±11 3 | 30.2±4 5 | 38 4±8 0 | 51 0±4 4 |
| (b) late-clamped | 12 | 84 2±12 3 | 42.0±5 3 | 41 9±9 5 | 57.5±5.5 |
| p-value | | <0 005 | <0 001 | — | <0 005 |
| IV Full term less than 2500 g | | | | | |
| (a) early-clamped | 6 | 80 5±11 8 | 35 7±8.2 | 45 0±4 1 | 50 0±5 1 |
| (b) late-clamped | 4 | 101 0±25 5 | 53 0±9 7 | 48 8±16 2 | 60 4±3 9 |
| p-value | | — | <0 05 | — | <0 01 |

plasma volumes were correspondingly plotted against the time of Volemetron determination no significant difference in the blood and red cell volumes could be demonstrated between those done within 1 hour from those between 1½ hours to 4 hours There was a suggestion of difference in the plasma volumes though not statistically significant

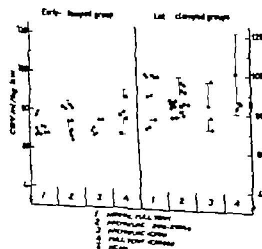



Fig 1 Scattergram of corrected blood volumes of newborn infants following early and late cord clamping

The predominant prenatal complications appeared to be threatened abortion and toxemia of pregnancy They occurred more frequently in the Group III and IV than in Group II infants (Table 1) Furthermore there was proportionately a higher incidence of prenatal complications in cases that developed IRDS 5 of 8 IRDS cases in Group III early clamped and 3 of 6 IRDS cases in the Group III late clamped had history of prenatal complications Threatened abortion was the main complica




Fig 2 Scattergram of red cell volume of newborn infants following early and late cord clamping

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infant in the corresponding clamping groups When cord clamping was delayed the red cell volume/kg b w of these under 2001 g infants appeared significantly smaller ($p < 0.05$) Since the red cell volume represents a less variable measure of the placental transfusion the present findings did not confirm those by others (2 4 10 18 20) who reported a relatively larger blood volume per kg b w in premature infants Our findings suggest that a relatively small vascular capacity in the premature infant (6) may to a large extent determine its blood volume and the amount of placental transfusion it received (7 8 1) With the high morbidity in Group III the blood volume may not represent that of normal premature infants However analysis of results leaving out those that were sick did not make any difference

The late-clamped premature infant seems to readjusted to its increased blood volume by plasma transudation into the extravascular space within the first hour of life like the full term infants as indicated by almost the same plasma volume per kg b w in both the early and the late clamped groups (5 13 16 17 21)

The clinical course and outcome of the truly premature cases were interesting (*Table 3*) Morbidity was expectedly higher in the smaller and more premature infants in group III There was similar incidence of IRDS in both early and late clamped cases The infants with birth weights closer to 2000 g tended to follow a normal clinical course Low Apgar scores were present more in those with lower birth weights though fairly scattered in the early clamped group (*Fig 3*) Low scores were present in most infants who developed IRDS Mortality appeared to be higher in the late clamped Group III cases in spite of the fact that most early-clamped of Group III started out in poorer condition with lower Apgar scores The deaths in the early-clamped were those of smallest birth weight The deaths in the late clamped group were more scattered in their weights The intensive initial cleaning of the airways and resuscitative procedures probably contributed to the favorable outcome in the early clamped But it cannot completely explain the higher mortality from IRDS in the late clamped who started out in a better general condition at birth

The high incidence of prenatal complications especially in the more premature infants (Group III) with IRDS probably bears causal relationship with prematurity itself as there was no significant difference in the prenatal complications among those that recovered or those that died of IRDS

This result differs from previous report claiming higher incidence and mortality from IRDS in early-clamped prematures (3 14) However it should be pointed out that those studies are not strictly comparable as there was no uniformity in defining early and late cord clamping And in those where blood volumes were determined they were carried out mostly at a later time than the first two hours after delivery or without simultaneous early and late clamped groups in the same study (3 4 9 10 11 14 18 19) In this connection it may be worth mentioning that studies on the respiratory mechanics of the early and late clamped full term infant (15) showed greater functional residual capacity and lung compliance in the early-clamped than in the late clamped and there was earlier slowing of the respiratory rate in the early clamped It is probable that the premature infant may respond to placental transfusion in a similar way

## SUMMARY

Blood volume was measured in 73 truly premature and 10 small for date term infants following early or late cord clamping at birth Forty five normal full term infants from a previous study were included for comparison The results demonstrated that placental transfusion occurred if cord clamping was delayed The amount of placental transfusion in terms of red cell volume per kg b w in the premature infants under 2001 g was less compared to that of both normal full term infants and premature

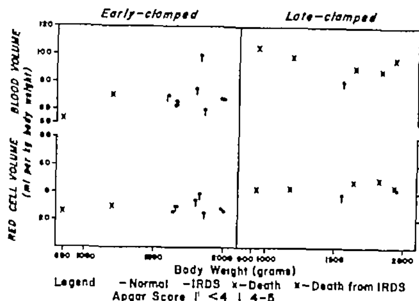



*Fig 3* Scattergram showing blood volume and red cell volume (RCV) of 17 early clamped and 12 late clamped premature infants weighing less than 2001 g with their clinical course and outcome

tion in these IRDS cases Both of the 2 deaths in Group III *a* 1 of 5 deaths in III *b* and 1 of 3 deaths in II *b* had a prenatal history of threatened abortion

The clinical course of the truly premature infants revealed that there were higher morbidity and mortality in infants weighing less than 2001 g Although the incidence of IRDS was about the same in both early and late clamped groups the mortality appeared higher in the late clamped, less than 2001 g group (Fig 3) This was impressive since the early clamped infants were in poorer condition at birth with lower Apgar score (Table 3) There was a tendency to clamp the cords early in these infants so that they could be resuscitated

The blood red cell and plasma volumes of the small for date infants appeared higher per kg b w than those of the infants in other groups but because of insufficient number of small for date infants, no significant statistical evaluation can be made

## DISCUSSION

It was clearly demonstrated by the results that if the cord clamping were delayed for 1 to 3 minutes or more the premature infant received a placental transfusion

We found no statistically significant difference in the blood volume per kg b w of the premature infant below 2001 g from those above 2001 g and from the normal full term

Table 3 *Clinical course and outcome of premature infants studied*

| Groups | Total No cases observed | Cases with clinical IRDS | Deaths | Apgar score < 6 |
|---|---|---|---|---|
| Truly Premature less than 2001 g | | | | |
| (*a*) early clamped | 17 | 8 | (2) | 10[a] |
| (*b*) late-clamped | 12 | 6 | 6 (5) | 2 |
| Truly Premature 2001–2500 g | | | | |
| (*a*) early clamped | 18 | 3 | (2) | 2 |
| (*b*) late clamped | 29 | 6 | 3 (2) | 1 |

Cases in ( ) with postmortem findings of pulmonary atelectasis varying degree of hyaline membrane disease and pulmonary hemorrhages

[*] One case with cerebral hemorrhage subdural left

One case with clinical IRDS no autopsy done

[a] Six cases with Apgar score less than 4

*Acta Pædiat Scand 58 567–571 1969*

# CONTROL OF RESPIRATION IN NEWBORN BABIES

## *I The Development of the Hering Breuer Inflation Reflex*

GÖRAN BODEGÅRD GÖSTA H SCHWIELER, STEN SKOGLUND
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BOYS GIRLS

*Fig 1* Birth weights and lengths of the infants studied plotted against weeks of gestation according to Swedish standards (9)

A

B

*Fig 2* Tracings of the transpulmonary pressure swings during spontaneous breathing in a baby of a postmenstrual age of 32 weeks (A) and in another of a postmenstrual age of 37 weeks (B)

The length of the breathing cycle before the occlusion is indicated by the distance *a* and that after the occlusion of the airway by the distance *b* The transpulmonary pressure at the moment of occlusion is indicated by the distance *c* The airway occlusion is indicated as a shift in level of the lower tracing The velocity is 25 mm in one second

regarded as the transpulmonary pressure at the moment of occlusion and thus as the pressure which simultaneously stimulates the pulmonary stretch receptors Only those experiments in which the occlusion was performed at the height of inspiration i e when the stimulation could be considered to be maximal have been included The pressures were recorded via two polyethylene catheters filled with saline One of them was introduced to the lower third of the oesophagus via one of the nostrils the other was ending in the rubber face mask The catheters were connected to a differential pressure transducer (T Ljungstom Sweden) and the pressure changes were recorded on a mingograph recorder (Elema Schonander Sweden)

In each baby some 10–15 acceptable occlusions were made Since the face mask adds to the dead space of the airways it was kept to the face for only a few minutes each time

Table 1 *Postmenstrual and postnatal ages of the nine babies examined*

Seven babies were examined once one was examined twice and one four times

| Case number | Postmenstrual age weeks | Postnatal age |
|---|---|---|
| 1 | 37 | 4 weeks |
| | 37½ | 4½ weeks |
| 2 | 34 | 1 week |
| 3 | 32 | 12 hours |
| 4 | 43 | 3 days |
| 5 | 36½ | 12 hours |
| 6 | 41 | 12 hours |
| 7 | 40 | 5 days |
| 8 | 39½ | 2 days |
| 9 | 32½ | 8 days |
| | 32¾ | 10 days |
| | 33¼ | 2 weeks |
| | 36¼ | 5 weeks |

## RESULTS

Tracings of the transpulmonary pressure and the respiratory rhythm in two typical experiments with occlusion of the airway at the height of an inspiration are shown in Fig 2 The occlusion caused a pronounced decrease of the respiratory rate in the 37 weeks old infant (B) whereas the rate remained unchanged in the 32 weeks old infant (A) The percentage change of the length of the breathing cycle after occlusion in relation to the transpulmonary pressure at a maximal inspiration immediately before the occlusion in the same two babies is shown in Fig 3 In the 37 weeks old baby an increase of the transpulmonary

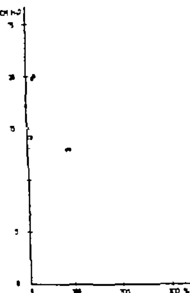


*Fig 3* Diagram showing the relative change in the length of the breathing cycle (abscissa) following airway occlusion at different transpulmonary pressures (ordinate)

The open circles derive from experiments on a baby of a postmenstrual age of 32 weeks, the filled from those on a baby of a postmenstrual age of 37 weeks.



*Fig 4* Diagram showing the mean percentage increase of the length of the breathing cycle (ordinate) following airway occlusion as plotted against the postmenstrual age (abscissa) The open circles derive from one and the same baby who was examined at different ages. The filled circles derive from the 8 other infants studied one of these babies was examined twice

pressure was followed by a prolongation of the breathing cycle whereas no such correlation was found in the 32 weeks old baby

The mean increase of the duration of the first breathing cycle following occlusion of the airway in relation to the postmenstrual age in all nine infants studied is shown in Fig 4 According to the results the Hering Breuer inflation reflex is very weak at a postmenstrual age of 32 weeks its strength then gradually increases until 36 to 38 weeks and then decreases

## COMMENTS

The Hering Breuer inflation reflex is known to participate in the regulation of the respiratory depth and frequency (1 4 14) but the full physiological significance is as yet not completely understood In several animal species the reflex is present at birth (18) Since newborn cats and rabbits develop periodic or gasplike breathing pattern following vagotomy which is not the case in later life (16) the reflex may be of particular importance in these animals during this period

In agreement with earlier observations (15) we have found that the Hering Breuer inflation reflex is present in newborn infants Our study shows an increase in the strength of the Hering Breuer inflation reflex up to 38 weeks of postmenstrual age and then a decrease (Fig 4) and it can be concluded that the increase and decrease in the strength of the reflex if such a development actually occurs with increasing maturity in utero is not interrupted by birth Our babies were studied at considerably varying times from birth (Table 1) and still seem to follow the same reflex development curve (Fig 4)

In the adult animal a decrease of lung compliance causes an increased discharge from the stretch receptors of the lungs (19) Since compliance increases with gestational age (8) the stimulus on the receptors would be more intense in babies of low gestational age than in those born at term The finding that the Hering Breuer inflation reflex is very weak at an early postmenstrual age may imply that the afferent and/or the central and/or the efferent linkages of the reflex fail Since it has been demonstrated that the maturation of the

peripheral nerve fibres as examined by morphological techniques is crucial for the development of different somatic reflexes (17) it may be of interest to study the nerve fibres involved in the Hering Breuer reflex in autopsy material from newborn babies of various gestational age

The view that respiration in the newborn baby is regulated in the same way as in adults is mainly based on qualitative observations (12) or on correlation of total ventilation to oxygen uptake (6) The fact that the net result in these respects is the same in the newborn as in the adult does not necessarily mean that the different regulation mechanisms have the same relative importance at different developmental stages According to the results of our study the strength of the Hering Breuer inflation reflex varies with postmenstrual age

It is interesting to speculate upon if there is any relationship between the weak Hering Breuer inflation reflex as found in infants of low postmenstrual age and the periodic breathing which frequently occurs in premature babies (7) The fact that vagotomy in newborn cats and rabbits is followed by a breathing pattern (16) which is similar to that seen in babies of low postmenstrual age speaks in favour of such an association The brainstem of those animals is deprived of the normal vagal afferent input from the lungs which is necessary for proper functioning (16) In analogy periodic respiration in babies of low postmenstrual age might be explained by insufficient thoracic afferent input to the brainstem respiratory center leading to a weak Hering Breuer inflation reflex

A possible explanation of the decline in strength of the Hering Breuer inflation reflex seen after the 38th postmenstrual week might be that other reflexes such as e g those from the thoracic wall then are becoming relatively more important

## SUMMARY

The Hering Breuer inflation reflex has been studied in babies of varying postmenstrual ages The strength of the reflex was assessed by relating the relative increase of the length of the breathing cycle to the transpulmonary pressure when the airway was occluded

The Hering Breuer inflation reflex was found to be very weak at a postmenstrual age of 32 weeks It was then found to increase to a maximum strength at a postmenstrual age of 36 to 38 weeks Later on there was a decline of the strength of the reflex

The significance of the findings in relation to the frequent occurrence of periodic and irregular respiratory rhythm in infants of low gestational age has been discussed

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# APPARENT RESPONSE OF IMPAIRED MENTAL DEVELOPMENT MINOR MOTOR EPILEPSY AND ATAXIA TO PYRIDOXINE

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In a review of the treatment of juvenile epilepsy with pyridoxine Hansson & Hagberg (6) suggested the term pyridoxine responsive seizures for attacks in elderly epileptic children who respond to the administration of pyridoxine despite an adequate dietary intake of the vitamin

A patient apparently belonging to this group is described The clinical and laboratory findings typical of the pyridoxine deficiency syndrome the pyridoxine dependency syndrome pyridoxine responsive seizures and the present case are summarized in Table 1

## REPORT OF CASE

A boy born October 28 1962 the third child of healthy non related parents Two sisters born in 1960 and 1961 are healthy The mother had had a few grand mal seizures of unknown cause at the age of 18 years She has then remained seizure free and apparently healthy without treatment Her electroencephalogram recorded in 1965 showed nothing remarkable No convulsive disorders were known in any other member of the family Both parents were 32 years old at the birth of the boy

The boy was born at term after a normal pregnancy Delivery was normal and the neonatal period was uneventful Birth weight was 4060 g Psychomotor development during the first year of life was normal The child started to walk at 1 year but always walked a little unsteadily No complications after vaccination with triple polio and smallpox vaccin Until his present disease he had been healthy He had had no cerebral trauma or infection

At 18 months of age (May 1964) slight convergent strabism was noticed The squint increased during the following months and glasses were ordered When the boy was almost 2 years (September 1964) his parents observed that his phychomotor development was retarded During the following months he showed a continuously increasing motor hyperactivity and lack of concentration From the age of 2 years and 4 months (February 20 1965) the patient had daily series of seizures with nodding of the head or a sudden fall forwards with jerky movements especially on the right side The seizures lasted only a few seconds and were not followed by loss of consciousness He had anything up to ten or twelve seizures in a few minutes

On admission (March 3 1965) the boy appeared physically normal with a weight of 14 7 kg and a height of 92 cm General physical examination revealed nothing remarkable At neurological examination a severe cerebral disorder was suspected He was tired anxious unconcentrated and restless It was difficult to establish rapport with him He had severe ataxia with a staggering gait and was prone to fall His hands were clumsy and he did not use the pinching grasp His vocabulary was small General muscle strength and tone were normal The muscle reflexes were normal as were the plantar responses no persisting neonatal reflexes could be elicited Examination of the cranial nerves showed severe convergent strabism without signs of bilateral paralysis of the sixth nerve The pupils reacted normally to light

Psychological examination according to Gesell (chronological age 2 5/12 years) confirmed the impression of a considerable mental retardation In motor and adaptive areas he had full scores up to the 15 month level At higher levels he scored well for his chronological age in some motor tests and in some adaptive tests even at the 2 year level Verbally he managed all tests for the 14 month level but none at the 18 month level In the personal social area he did not manage any tests beyond the 21 month level

*Laboratory studies* Routine analysis of the blood

Table I *Clinical and laboratory findings in patients with pyridoxine deficiency, pyridoxine dependency and pyridoxine responsive seizures*

| | Deficiency | Dependency | Responsive seizures | Present case |
|---|---|---|---|---|
| t onset | Infancy | Neonatal period | Any age | 2½ years |
| ...tus, ceased r pyridoxine | Yes | Yes | Yes | Yes |
| ...al retardation | May develop if condition is left untreated | Always develops if condition is left untreated | Is often though not always present when diagnosis is established | Yes |
| ...n | Difficult to evaluate | Impossible to evaluate | May be present | Yes |
| abnormalities | Yes | Yes | Yes | Yes |
| ...rmal haematolog findings | Often | No | No | No |
| ...no acids in urine | Increased after pyridoxine | No changes | | Increased after pyridoxine |
| ...thic acid in urine | High before pyridoxine, decreased after | No changes | | No changes |
| ...ree in urine | Increased after pyridoxine | No changes | | Increased after pyridoxine |
| ...ptophan load test | Abnormal | Normal | Variable | Normal |

...urine revealed nothing remarkable. The cerebrospinal fluid contained no cells. The total protein content was 36 mg per 100 ml and the electrophoretic pattern was normal. CSF sugar was 60 mg per 100 ml (blood sugar 70 mg per 100 ml) and chlorides ...3 mEq per litre. The serum concentrations of calcium, phosphorus and alkaline phosphatases were within normal limits. The Wassermann reaction and tests for listeria and toxoplasmosis antibodies were negative. Analysis of amino acids in urine showed a normal spectrum. A tryptophan load test (0.2 g tryptophan per kg bodyweight) was normal. An X-ray of the skull, pneumoencephalogram and echoencephalogram revealed nothing remarkable.

An EEG the day after admission was abnormal with a large amount of episodic slow spike and wave activity with high voltage and bilateral synchronicity.

*Clinical course.* During an initial period of investigation and observation, the boy had daily repeated seizures lasting only a few seconds: he threw his arms out, lost muscle tone and fell forwards without loss of consciousness. After a fortnight he was given 120 mg of pyridoxine and 7.5 mg of Valium® a day by mouth. Valium® was withdrawn after two weeks, and the pyridoxine after four weeks. Two weeks later it was resumed and has since been given. During the withdrawal period amino acid studies and EEG-examinations were performed.

The day after the first dose of pyridoxine and Valium® the convulsions disappeared completely and did not recur on withdrawal of Valium® and subsequent withdrawal of pyridoxine for two weeks. An EEG recorded after ten days' treatment with pyridoxine and Valium® showed no paroxysmal discharges but considerable fast activity, probably due to Valium®. Later control EEG recordings revealed transient hypersynchronous fast paroxysmal spindles on one occasion. At the last examination (July 1968) there were no paroxysmal abnormalities. The background activity was somewhat slow for his age.

The patient had no convulsions during the later course. His gross motor and mental development has shown a slow, steady improvement. At neurological examination in July 1965 (age ... years and 9 months) he still had a squint, but otherwise showed nothing remarkable. There was no ataxia. He was still somewhat anxious and restless, and his speech was retarded. At examination two years later (July 1967) he was still on 170 mg pyridoxine a day. His mental capacity and speech were better, it was easy to establish rapport with him, and his behaviour appeared normal for his age. A Terman Merrill test gave an IQ of 89 and a Goodenough drawing was adequate for his age.

The pyridoxine dose has since been gradually decreased to 40 mg a day. At the last examination (August 1968) his physical condition was excellent and the neurological routine examination revealed nothing abnormal except strabism. His mental development was normal for his age (Terman Merrill test, IQ = 96) and he was emotionally well adapted.

## LABORATORY STUDIES

### *Methods*

The laboratory studies included not only routine examinations but also measurement of the urinary α-amino nitrogen by the method of Khachadurian *et al*

(7) Urinary free amino acids were separated by combined high voltage electrophoresis and chromatography principally in the way described by Kickhofen & Westphal (8) The apparatus described by Wieland & Pfleiderer (15) was used

Urine samples were initially passed through a 1×3 cm column of Zeokarb 225 resin to reduce volume and remove urea (4) The amino acids were separated under the following conditions formate acetic acid buffer pH=1 2 voltage gradient 50 V per cm running time 90 minutes Whatman No 3 MM filter paper Further resolution of the amino acids was achieved by ascending paper chromatography in a direction perpendicular to that of the proceeding electrophoresis A mixture of glacial acetic acid n butanol and water (1 4 5 v/v) was used as solvent Taurine was then evaluated quantitatively by staining with ninhydrin and afterwards with copper reagents (5) The spots were then eluted from the filter paper with methanol and measured spectrophotometrically at 504 nm The calculation was based on appropriate standards and blanks The accuracy of this method was about ±10

The measurement of urinary xanthurenic acid after the tryptophan load test was done as described by Maske (9) The child received 0 2 g 1 tryptophan per kg bodyweight

## RESULTS

After three days treatment with pyridoxine (120 mg per day) the urinary excretion of α amino acid nitrogen increased twofold to 286 μg/mg creatinine and the urinary excretion of taurine increased twenty fold to 2 2 μg/mg creatinine Two days later the α amino acid nitrogen level was almost unchanged (265 μg/mg creatinine) but the taurine excretion started to decrease (1 7 μg/mg creatinine)

Twelve days after the beginning of treatment with pyridoxine the excretion of taurine was again normal The excretion of urinary amino acids continued to increase to reach a level of 387 μg/mg creatinine after two months treatment with pyridoxine The taurine excretion was then 0 06 μg/mg creatinine (Table 2)

Re examination of the urine during the period when no pyridoxine was given revealed no change in the urinary excretion of amino acids or taurine

The distribution of the urinary amino acids was found to be normal in all examinations except immediately after pyridoxine therapy had been started, when the above mentioned

Table 2 *Taurine excretion*

All values are given in mg per g creatinine The patient received pyridoxine (120 mg daily) between March 2[illegible] and April 22 and continuously after May 6

| Date | Urinary α amino N | Urinary taurin |
|---|---|---|
| 3 3 | 174 | 0 11 |
| 24 3 | 286 | 2 20 |
| 26 3 | 265 | 1 70 |
| 2 4 | 295 | 0 12 |
| 29 4 | 302 | 0 12 |
| 5 5 | 329 | 0 10 |
| 26 5 | 387 | 0 06 |

temporary increase of the taurine excretion was noted No urinary cystathionine (detection limit 0 2 μg/mg creatinine) could be demonstrated before or during pyridoxine therapy

## DISCUSSION

When first seen this boy showed signs of a severe progressive cerebral disorder with minor motor epilepsy, ataxia and mental retardation Neither the patient s history nor clinical investigations suggested a prenatal or perinatal cerebral injury A metabolic cause was therefore suspected

The clinical response to pyridoxine was dramatic There was no recurrence of the convulsions when Valium® was withdrawn The ataxia disappeared and the child s behaviour improved and continued to do so slowly and steadily for at least a few years Withdrawal of treatment to check whether the improvement was due to the pyridoxine was refrained from it being feared that this might jeopardize the favourable mental development of the child

It is well established that pyridoxal phosphate is important to normal brain function The relation between seizures in childhood and pyridoxine is complicated The pyridoxine deficiency syndrome and the pyridoxine dependency syndrome are well delimited entities (6 14) in which pyridoxine plays an unequivocal role There is, however a third ill defined group of epileptic children in whom the disease appears to respond to pyridoxine (6)

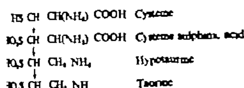



Fig 1 Cystine metabolism. Decarboxylation of cysteine sulphinic acid requires presence of pyridoxal phosphate

though usually less dramatically than in our patient. In some patients reported to have pyridoxine responsive seizures withdrawal of pyridoxine has not been followed by subsequent recurrence of the symptoms This group of patients is thus heterogenous and no metabolic disorder common to all of them has yet been demonstrated

The transient increase of urinary taurine excretion during pyridoxine therapy was the most remarkable biochemical finding in our patient A similar change in taurine excretion has been reported by Scriver & Hutchison (11) in a 14 month old child with a vitamin $B_6$ deficiency syndrome

Taurine is a metabolite of cysteine and is formed by decarboxylation of cysteine sulphinic acid with hypotaurine as the intermediary product (Fig 1) This decarboxylation requires the presence of pyridoxal phosphate Blaschko *et al* (3) and Bergeret *et al* (1) showed that the urinary excretion of taurine ceased in rats rendered $B_6$-deficient but reappeared on administration of pyridoxine The change in taurine excretion in our patient is presumably related to an increased pyridoxine requirement Some degree of pyridoxine deficiency is possible though less likely because unlike Scriver & Hutchison's patient, our patient had no cystathioninuria and reacted normally to tryptophan loading The possibility of a pyridoxine deficiency cannot, however be ruled out since various enzymes active only in the presence of pyridoxal phosphate differ considerably in their affinity to this coenzyme (12) This implies that not all pyridoxine-dependent enzymes will be affected to the same degree by pyridoxine deficiency The possibility of simple dietary pyridoxine deficiency first reported by Snyderman *et al* (13) was excluded in our case

Berlow (2) found the urinary excretion of taurine in a patient with cystathioninuria to be slightly increased during pyridoxine treatment He regarded this taurinuria as a sign of increased cystathionine metabolism

Urinary excretion of taurine did not change in 5 healthy children when given pyridoxine (80 mg daily) by mouth (unpublished personal observations)

Another similarity between our patient and Scriver & Hutchison's was the increased excretion of amino acids in general during treatment with pyridoxine No explanation for this increase can be offered since we did not measure the plasma amino acid levels The markedly increased excretion of taurine (about 20 times pretreatment value) in the beginning of pyridoxine treatment was only transient The general aminoaciduria however lasted for more than 2 months and resulted in a twofold rise of the pretreatment value

An abnormal response to the tryptophan load test can be expected only in patients with the pyridoxine deficiency syndrome In the pyridoxine dependency syndrome it is invariably normal these patients can be recognized only by the dramatic response to pyridoxine therapy In pyridoxine responsive seizures the result of the tryptophan load test is variable The test is often unreliable as it is influenced by exogenous factors such as antiepileptic drugs (10) which may produce an abnormal response in children who do not benefit from the administration of pyridoxine At present neither pyridoxine dependent nor pyridoxine responsive patients can thus be identified on clinical ground or by laboratory studies The only method available is a therapeutical trial with pyridoxine Such a trial is clearly indicated in all cases of neonatal seizures it is also justified in older children with obscure minor motor epilepsy mental retardation or deterioration and progressive neurological signs Our patient probably had an in

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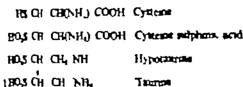



*Fig 1* Cysteine metabolism Decarboxylation of cysteine sulphinic acid requires presence of pyridoxal phosphate

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creased pyridoxine requirement manifested clinically by impaired mental development minor motor epilepsy and ataxia and biochemically by impaired taurine metabolism

## ACKNOWLEDGMENT

We thank Dr D Ingvar Dept of Neurophysiology University of Lund for valuable help with the interpretation of the electroencephalograms

## SUMMARY

Impaired mental development, minor motor epilepsy and ataxia in a 2 1/ year old boy without cystathioninuria and with a normal reaction to the tryptophane loading test responded favourably to pyridoxine Transient taurinuria and a long lasting general aminoaciduria occurred during the treatment Certain clinical and biochemical findings and observations suggested that the child s symptoms were due to an increased pyridoxine requirement

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# THE FOETAL DEVELOPMENT OF SERUM LEVELS OF IgG AND IgM

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The immunoglobulin in the newborn baby consists mainly of maternal IgG The capacity for immunoglobulin synthesis exists however already during foetal life The newborn baby practically always shows IgM in the serum (1 2 6 11) This arises predominantly from the child itself since IgM does not normally traverse the placenta in any noteworthy amount (9) Intrauterine infections seem to be able to stimulate the foetus to production of both IgM and IgA (5 9) Foetal synthesis of IgG has also been reported though of a quantitatively minor importance (7 15 17)

It has long been known that during early pregnancy the foetus has a considerably lower protein level in the serum than the mother both as regards albumin and gamma globulin (3) Full term infants on the other hand have on the average a distinctly higher serum concentration of IgG than their mothers (1 13) which is regarded as evidence of active placental transport of IgG

It is also well known that during the early part of pregnancy the foetus has lower titres of certain antibodies e g antistreptolysin and antistaphylolysin than the mother while during later pregnancy the foetal and maternal titres of these antibodies are similar (21)

Recently published investigations have shown a good correlation between serum concentration of IgG in the prematurely born infant at birth and gestational age (2 10 22) Prematurely born infants have lower serum concentrations of IgG than full term newborns and infants born very prematurely can have very low serum IgG concentrations at birth

Foetal synthesis of IgM has been reported from about the 20th week of gestation (7 15) In a previous study no markedly lower serum concentrations of IgM at birth were found in premature than in mature infants Extremely premature infants however showed a clearly retarded IgM development during the first weeks of life compared with more mature infants (2)

The aim of the present study was to shed further light on the development of the serum concentrations of IgG and IgM during foetal life

## MATERIAL AND METHODS

The material consisted of 21 non viable foetuses (including one pair of twins) from legal abortions performed during the 13th 26th week of pregnancy 35 infants (including one pair of twins) born before the estimated time i e during the 26th–37th week of pregnancy 14 infants born at the estimated time (during the 39th–42nd week of pregnancy and 7 infants born after the estimated time (during the 43rd–45th week of pregnancy) The series of prematurely born infants was collected intentionally in such a way that it showed a clear overrepresentation of infants of very short gestational age

The abortions were performed by abdominal hys

terotomy on social or psychiatric indications In all cases the amniotic membranes appeared to have been intact before the hysterotomy No external malformations or other obvious abnormalities were found on inspection of the foetuses Even in the absence of clinical suspicion of intrauterine infection however such an infection cannot definitely be excluded in any of these cases (nor in any of the viable infants)

In most of the non viable foetuses the blood samples were taken as follows Before detachment of the placenta the umbilical cord was clamped off and carefully cleaned of maternal blood The umbilical cord was severed on the placental side of the clamp which was then removed after which the foetal blood was collected in glass tubes In this way blood volumes of about 1-2 ml were obtained In one case foetal blood was taken by cardiac puncture and in another case via an umbilical vein catheter Blood samples from the mother were taken preoperatively by venepuncture In all prematurely born and mature infants the blood samples consisted of umbilical cord blood Blood samples from the mothers of these infants were taken at delivery by venepuncture

In 7 cases blood samples were taken only from the foetus or infant and not from the mother

In a further 9 mature newborn infants both umbilical cord and capillary blood samples were taken immediately after birth and a further capillary sample was taken 2 hours postnatally

The blood samples were centrifuged after coagulation and the serum was removed by pipetting and stored at −20 pending analysis The immunoglobulin determinations were performed by means of single radial immunodiffusion in agar gel according to the method of Mancini *et al* (14) with certain modifications (12)

The length of gestation was calculated from the first day of the last normal menstruation In some cases where this information was uncertain consideration was also taken in the calculation of the size of the uterus and of the mother's observations of foetal movements In general there was a good agreement between gestational age on the one hand and length at birth and birth weight on the other in the foetuses and infants investigated In the series no markedly "small for dates" infants were included The lowest birth weight in the infant of gestational ages exceeding 42 weeks was 3000 g None of those infants showed pronounced signs of placental dysfunction

The statistical calculations were performed on both arithmetic values and on values transformed logarithmically to base 10 IgM values less than 1 mg/100 ml were calculated as 0 5 mg/100 ml in the statistical analyses With one exception (the correlation between the maternal serum IgG concentration and length of gestation) only the results of those regression analyses which were performed on the logarithmic values are presented in the figures and the table

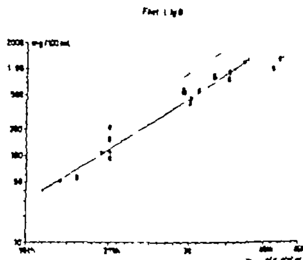


*Fig 1* IgG concentrations in umbilical cord serum in foetuses and in premature mature and postmature infants Regression lines for the IgG development (log IgG on gestational age) up to and including the 37th week of pregnancy (——) and between the 35th and 42nd weeks of pregnancy ( ) 95% confidence interval (- - -)

## RESULTS

IgG was found in the serum of all foetuses and infants studied The lowest IgG concentration 29 mg/100 ml was observed in a foetus in the 13th week of gestation while full term newborns showed on the average an IgG concentration of 1416 mg/100 ml There was a clear relationship between the IgG concentration in the foetuses and infants and gestational age The logarithmic IgG values showed a considerably better correlation to gestational age than the arithmetic values

As can be seen in Fig 1 the serum concentration of IgG in the foetus increased exponentially during pregnancy with the exception of its last few when the development was relatively slower The best correlation between the logarithmic IgG value of the foetus and gestational age was obtained on comparison up to and including the 37th week of pregnancy ($r = 0.940$)

There was some correlation between the foetal and maternal IgG levels Multiple regression analysis showed that the serum IgG concentration of the mother was of significant importance for the foetal IgG value ($p < 0.025$) A somewhat lower correlation was obtained

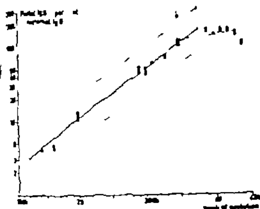


Fig 2 The development of the foetal maternal IgG ratio during pregnancy Regression lines (log foetal IgG maternal IgG 100 on gestational age) up to and including the 37th week of pregnancy (——) and between the 35th and 42nd weeks of pregnancy ( ) 95 confidence interval ( -)

on comparison within the group of full term newborns alone but here also a relationship was found between the IgG concentrations in the mother and infant ($p \approx 0.05$)

The ratio of foetal maternal IgG concentration was highly correlated to the length of gestation and like the foetal IgG concentration increased exponentially during pregnancy with the exception of the last few weeks (Fig 2) The best correlation ($r = 0.957$) between the logarithmic value for the foetal maternal IgG ratio and the gestational time was found on comparison up to and including the 37th week of pregnancy The foetal maternal IgG ratio was increased tenfold from the 20th to the 35th weeks of pregnancy Premature infants with a gestational age of 35 weeks had on the average somewhat higher IgG concentrations than their mothers and full term newborns showed on the average just over 150 % of the maternal IgG level High infant maternal IgG ratios were found in some cases where the mothers had low serum concentrations of IgG One mature infant whose mother had an IgG concentration of only 403 mg/100 ml thus had 272 of the maternal IgG level (1095 mg/100 ml

As shown in Fig 3 the mean maternal serum concentration of IgG decreased during pregnancy This decrease was statistically significant ($p < 0.001$)

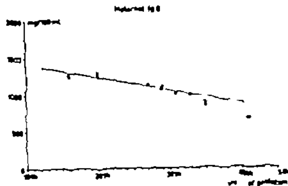


Fig 3 Maternal IgG concentrations Regression line (IgG on length of gestation) up to and including the 40th week of pregnancy (——)

As is evident from Fig 4 the foetal maternal IgG ratio was well correlated to the foetal length especially during the early part of pregnancy On comparison within the group of premature infants alone it was found that the serum concentration of IgG like the infant maternal IgG ratio was better correlated to the gestational age than to the birth weight and the length at birth

In those infants from whom capillary samples were taken immediately after birth simultaneous with umbilical cord samples higher IgG concentrations were found in all cases in the capillary sera than in the umbilical cord sera the former level being on the aver

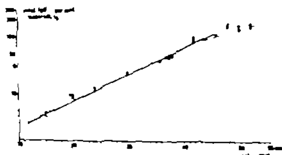


Fig 4 Comparison between the foetal maternal IgG ratio and foetal length Regression line (log foetal IgG/maternal IgG 100 on foetal length) up to and including a foetal length of 45 cm (——) and from a foetal length of 42 cm ( )

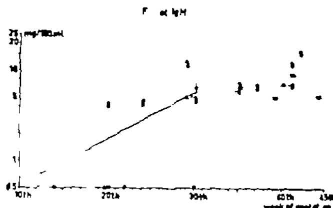


*Fig 5* IgM concentrations in umbilical cord serum in foetuses and in premature mature and postmature infants Regression lines for the IgM development up to and including the 30th week of pregnancy (——) and between the 30th and 45th weeks of pregnancy (      )

age 28% higher than the latter This difference was statistically significant ($p<0\ 001$) In the capillary sera taken 2 hours after birth somewhat higher IgG concentrations were observed on the average than in capillary sera taken at birth this difference was not statistically significant, however

IgM was found in the serum (Fig 5) in one foetus in the 14th week of pregnancy (3 9 mg/100 ml) and in one foetus in the 16th week (3 6 mg/100 ml) Among foetuses in the 20th week of pregnancy, IgM was found in 3 cases out of 6 and from the 24th week IgM was found in the serum in all cases except one (a premature infant with a gestational age of 30 weeks) Regression analysis showed that the IgM concentration in the serum rose significantly up to the 30th week of pregnancy ($p<0\ 005$) Between the 30th and 45th weeks of pregnancy some further IgM increase was noted, but this was not statistically significant

The numerical results of regression analyses corresponding to regression lines drawn in the figures are given in Table 1

## DISCUSSION

The finding in prematurely born infants of a linear relationship between the logarithmic value for the serum IgG concentration at birth and the gestational age was reported by Hobbs & Davis (10) As shown in the study presented

Table 1 *Numerical results of regression analyses corresponding to regression lines drawn in Figs 1–5*

| | | $S_e$ | $r$ | $R^2$ |
|---|---|---|---|---|
| Foetal IgG (mg/100 ml) } regression on gestational age | | | | |
| Foetal IgG (per cent of maternal IgG } | | | | |
| Gestational | log foetal IgG = 0 8768 + 0 0604 *t* ** | 0 1543 | 0 940 | 0 884 |
| age ~37 weeks | log $\frac{\text{foetal IgG}}{\text{maternal IgG}}$ 100 −0 3092 0 0669 *t** | 0 1432 | 0 957 | 0 917 |
| Gestational | log foetal IgG = 2 2257 + 0 0223 *t* * | 0 1021 | 0 531 | 0 282 |
| age = 35–42 weeks | log $\frac{\text{foetal IgG}}{\text{maternal IgG}}$ 100 1 6189 0 0131 *t* | 0 1297 | 0 279 | 0 078 |
| Maternal IgG (mg/100 ml) regression on length of gestation | | | | |
| Length of gestation ~40 weeks | IgG − 1600 86 − 17 98 *t*** | 266 76 | −0 451 | 0 203 |
| Foetal IgG in per cent of maternal IgG regression on foetal length | | | | |
| Foetal length ~45 cm | log $\frac{\text{foetal IgG}}{\text{maternal IgG}}$ 100 −0 0690 0 0469 *l** | 0 1441 | 0 958 | 0 918 |
| Foetal length ~42 cm | log $\frac{\text{foetal IgG}}{\text{maternal IgG}}$ 100 0 8229 + 0 0260 *l** | 0 1598 | 0 499 | 0 249 |
| Foetal IgM (mg/100 ml) regression on gestational age | | | | |
| Gestational age <30 weeks | log IgM = −0 8052 + 0 0527 *t* | 0 4256 | 0 580 | 0 337 |
| Gestational age >30 weeks | log IgM = 0 3493 + 0 0125 *t* | 0 2378 | 0 43 | 0 059 |

*t* = gestational age (or length of gestation)
*l* = foetal length The degree of significance is denoted by asterisks

here the same linear relationship exists also during an early stage of pregnancy but not on the other hand during the last few weeks of a normal pregnancy which latter has been claimed by Yeung & Hobbs (22)

In addition to the length of gestation the maternal serum IgG level also seems to influence the foetal IgG concentration even though to a considerably smaller degree That the serum concentration of IgG in the mother is of some importance for the corresponding concentration at birth in mature infants has been demonstrated by Allansmith *et al* (1) Bridges *et al* have described the gamma globulin development in a child of a woman with acquired agammaglobulinaemia (4) This child was born with a very low gamma globulin concentration in the serum and had gamma globulin concentrations below 11 mg/100 ml up to the age of 6 weeks That the maternal IgG level only has a limited influence on the level in the child is illustrated however by the finding of completely normal IgG concentrations in children of mothers with remarkably low IgG values

*B*ecause of the fact that there is some relationship between the maternal and foetal IgG levels a somewhat better correlation is obtained if instead of the foetal IgG concentration the foetal maternal IgG ratio is correlated to the length of gestation This mode of procedure has further the advantage that the results obtained can be compared without difficulty with those of similar investigations in which a different method or another immunoglobulin standard has been used

In Fig 2 the development of the logarithmic foetal maternal IgG ratio appears to comprise a linear function This seems to apply up to about the 35th–37th weeks of pregnancy but not after this time The five values which are found before the 18th week of pregnancy all lie below the regression line which may be due to chance It cannot be excluded however that an even better correlation between the foetal maternal IgG ratio and the length of gestation might be obtained if the development of the logarithmic value for the IgG ratio during pregnancy were presented as a curvilinear function

The calculation of the length of gestation can present difficulties in a study such as this and can constitute a source of error which might explain individual deviations from the otherwise so uniform tendency We have therefore also correlated the foetal maternal IgG ratio to foetal length Here also a very good correlation was obtained As can be seen in Fig 4 this holds especially for foetal lengths of less than 35–40 cm while at larger foetal lengths the scatter around the regression line is considerably greater This explains the relatively low coefficient of correlation obtained on comparison of the infant maternal IgG ratio with foetal length within the group of premature infants alone

Yeung & Hobbs (22) who compared 12 infants with gestational ages of 42 weeks or more who were defined as postmature with 30 infants with gestational ages of 40 weeks found considerably lower initial IgG concentrations in the former group We have not made the same finding the 7 infants with gestational ages between 43 and 45 weeks had practically equally high IgG concentrations at birth (mean value 1400 mg/100 ml) as the 14 infants with gestational ages of 39–42 weeks (mean value 1416 mg/100 ml) It seems reasonable however that changes in the placenta such as for example pronounced infarction leading to placental insufficiency might influence the transport of IgG from mother to foetus This is also confirmed by the finding by Yeung & Hobbs of low IgG levels at birth in small for-dates babies

The finding of a higher IgG concentration in capillary sera than in umbilical cord sera is not very surprising It is well known that in normal infants both the haemoglobin concentration and the plasma protein concentration increase rapidly after birth One essential reason for this is the blood transfusion which the child receives from the placenta and whose size can vary depending on how quickly after

delivery the umbilical cord is clamped off (16) Furthermore, in newborn babies distinctly higher haemoglobin concentrations have been found in capillary than in venous blood even when the samples have been taken at the same time Vahlquist (20) has thus shown that in newborn infants both at birth and at the age of 1 day, there is a significant difference between the haemoglobin concentration in capillary and venous blood A difference but not statistically significant was still observed on the 6th day of life whereas when the infants were 2 weeks old no such difference was found

In previous studies on the initial IgG concentrations in infants of varying gestational ages, either capillary blood alone (2) or alternating umbilical cord blood and capillary blood (10 22) have been used Owing to the systematically higher IgG levels in capillary blood these studies are therefore not fully comparable with those of the present investigation it is possible that divergent results can be explained partly by the differences mentioned in sampling techniques

The finding of a tendency towards lower IgG values in women during pregnancy agrees with the results found by Mac Gillivary & Tovey in 1957 (8) They showed by means of paper electrophoresis that the serum concentration of gamma globulin in the pregnant woman decreased gradually during pregnancy Paaby *et al* however found no significant change in the gamma globulin concentrations in women during pregnancy (18) It would seem of interest to follow the IgG levels longitudinally with a modern quantitative technique in women during pregnancy in order to obtain more definite information on this matter

The finding of IgM in the serum in two foetuses with gestational ages of 14 and 16 weeks respectively is of interest since as far as we know the occurrence of IgM in the serum of foetuses with such short gestational ages has not been described previously Contamination of the blood samples from these foetuses with maternal blood is, of course, conceivable but seems hardly probable partly in view of the sampling techniques and partly since the same sera from these foetuses contained low concentrations of IgG (63 and 58 mg/100 ml) Van Furth *et al* (7) who in 20 foetuses with gestational ages of 13-31 weeks examined splenic, thymic and lymph gland tissue and preparations from peripheral blood with immunofluorescent staining and also studied the immunoglobulin concentrations in serum found evidence of synthesis of IgM (and also IgG) from approximately the 20th week of pregnancy Matsen *et al* (15) studied bone marrow specimens from foetuses with gestational ages of 24-42 weeks and observed synthesis of both IgM and IgG in all cases

In the series presented here, no significant IgM increase in umbilical cord sera was observed from about the 29th-30th weeks of pregnancy and with increasing gestational age This agrees with previous findings in premature infants (2) In foetuses of shorter gestational age for some reason either well evident IgM concentrations (3 3-4 6 mg/100 ml) or no (<1 mg/100 ml) IgM at all were found


## SUMMARY

The serum concentrations of the immunoglobulins G and M were studied in non viable foetuses with gestational ages of 13-26 weeks and also in premature mature and postmature infants Among the prematurely born infants there was an overrepresentation of infants with very low gestational ages The serum IgG concentrations in the mothers at the time of abortion or delivery were also studied

In full term infants there was a systematic difference between simultaneously taken capillary and umbilical cord samples with higher serum concentrations of IgG in the former For this reason umbilical cord sera were used throughout in this study

The maternal serum concentration of IgG was found to be of some importance for the foetal IgG level The foetal maternal IgG ratio increased as also did the foetal IgG concen

tration, exponentially during pregnancy with the exception of its last weeks when the development took place relatively more slowly

IgM in serum was observed (>1 mg/100 ml) in two foetuses with gestational ages as short as 14 and 16 weeks From the 24th week of pregnancy onwards IgM was found in the serum in practically all cases From about the 29th-30th week of pregnancy no significant increase in the serum IgM with increasing gestational age was observed in the infants studied

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delivery the umbilical cord is clamped off (16) Furthermore in newborn babies distinctly higher haemoglobin concentrations have been found in capillary than in venous blood even when the samples have been taken at the same time Vahlquist (20) has thus shown that in newborn infants both at birth and at the age of 1 day there is a significant difference between the haemoglobin concentration in capillary and venous blood A difference, but not statistically significant was still observed on the 6th day of life whereas when the infants were 2 weeks old no such difference was found

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In full term infants there was a systematic difference between simultaneously taken capillary and umbilical cord samples with higher serum concentrations of IgG in the former For this reason umbilical cord sera were used throughout in this study

The maternal serum concentration of IgG was found to be of some importance for the foetal IgG level The foetal maternal IgG ratio increased, as also did the foetal IgG concen

*Table 1. Normal daily excretion of carnosine, histidine, 1 methylhistidine and β alanine in urine of children and adults reported by several authors*

| Author | Ref | Subjects | Total μmoles | | | |
|---|---|---|---|---|---|---|
| | | | Carnosine | Histidine | 1 Methyl histidine | β-Alanine |
| Bigwood (cited by Soupart) | 13 | 15 children | — | 99–533 | 0–250 | — |
| Block | 4 | 2 adults (meat free diet) | 59–61 | 509–775 | 19 5–26 6 | — |
| Evered | 5 | 3 adults | — | 625 | 130 | — |
| Gladstone | 6 | 6 adults | — | 970 | — | — |
| | | 4 children | — | 342 | — | — |
| Perry | 8 | 2 adults (regular diet) | 0 | 410–1200 | 245–615 | 0 |
| | | (steak diet) | 333–680 | 3650–4400 | 0 | 1060–1830 |
| | | (chicken breast diet) | 2940–4250 | 3590–3870 | 13 700–19 270 | 225–2890 |
| | | Children | Usually not detectable | — | — | — |
| [illegible]upart | 12 | 9 females | — | 825 | 384 | 33 8 |
| | | 6 males | — | 890 | 431 | 67 7 |
| [illegible] | 14 | 6 males | — | 1390 | 1060 | — |
| [illegible]ci | 15 | 44 adults | 15 9 | 1111 | — | — |
| | | 54 children | 9 3 | 928 | — | — |
| [illegible]stall | 16 | 1 adult | 8 8–13 3 | — | — | — |

— Not determined

[illegible] to the urine specimens collected outside the hospital.

To confirm the identity of carnosine and anserine [illegible] was subjected to hydrolysis in 6 N hydrochloric acid for 6 hours at 100°C. The resulting preparation was heated in a boiling water bath until dryness. The residue was dissolved in sodium citrate buffer (pH 2.2) and analyzed for histidine, 1 methylhistidine and β alanine. Carnosine and anserine could be identified by a complete disappearance of both peaks in the chromatogram of the hydrolyzed samples and by the increase of β alanine and either histidine or 1-methylhistidine.

Serum-carnosinase activity had been determined according to a procedure described by Perry et al (9).

The oral loading tests and the determination of serum-carnosinase activity were performed with L-carnosine which was obtained from Fluka AG, Buchs SG, Switzerland.

## RESULTS

Comparison of the urinary excretion of carnosine reported by several authors (Table 1) with that of our patient R. R. indicated that the latter excreted increased amounts of the dipeptide in the urine (Table 2) even if a meat free diet was given. From Table 1 it appeared that little is known about the normal excretion of carnosine in urine by children. Tocci & Bessman (15) investigated 54 children and they found a mean daily excretion of 9 3 μmoles of carnosine. Perry et al (8) reported that urine of normal children usually does not contain carnosine on regular diets.

Up till now several cases of increased carnosine excretion have been described (3, 7, 8). In these reports the value given by Westall (16) determined for only 1 adult male had been considered to be a normal carnosine excretion. The supposition that our patient excreted unusually large amounts of carnosine was initially based on this normal value which was known at the start of this investigation.

*Influence of diet on the carnosine excretion.* In order to establish if the increased carnosine excretion by our patient was from dietary origin he was fed a regular diet followed by a 5 day period in which the diet was essentially free of any carnosine. The result of this experiment is shown in Table 2. It can be seen that

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# A PATIENT WITH A DEFICIENCY OF SERUM CARNOSINASE ACTIVITY

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An increased urinary excretion of carnosine has been reported by Bessman & Baldwin (3) and by Levenson *et al* (7) in children suffering from cerebro macular degeneration Perry *et al* (8) described two patients suffering from a progressive neurological disease who also showed carnosinuria In the latter two cases elevated serum carnosine levels were found after oral loading with L-carnosine Furthermore little or no serumcarnosinase activity was observed (9)

The present study deals with a patient with increased urinary carnosine excretion and strongly decreased serum carnosinase activity

## CASE REPORT

R R (660085) born Oct 27th 1964 is a caucasian male of Dutch origin He is the first child of healthy consanguineous parents The patient was born spontaneously after an uncomplicated gestation of 37 weeks The birthweight was 3000 g In the neonatal period there were no complications except for a moderate icterus during the first week of life At the age of 6 months the parents noticed that the patient s eyes did not fix on a presented object He also had spells of opisthotonus A neurological examination at this age did not reveal any definite abnormalities but the patient was considered to be retarded in his mental development Opthalmologic examination at the ages of 1 1 4/12 2 4/12 3 4/12 and 3 9/12 years revealed a non progressive temporal paleness of the optic papillae The electroretinograms were always normal

At the age of 2 8/12 years the patient was admitted for clinical observation at the paediatric department The mental and motor development were extremely retarded all functions were below the normal performance for 40 weeks of age Except for a microcephaly (46 cm) the roaming eyes and an autistiform pattern of behaviour the physical examination did not reveal any abnormalities The electroencephalograms evolved from a dysrhythmic pattern at the age of 1 3/12 years via a distinct focus left occipitally at the age of 2 4/12 years to a deep situated focus manifesting itself chiefly in the left occipital region At the age of 3 10/12 years the patient showed generalized convulsions The electroencephalogram at this age showed spikes in the right parietal region and in the occipital regions Laboratory investigations revealed no abnormalities except for a marked carnosinuria The patient s brother born Oct 10th 1968 excreted no carnosine at the age of 12 days but a carnosinuria was observed during the 9th week of life (53 μmoles/24 h) Serum obtained at the age of 4 weeks showed no carnosinase activity

A family study is in preparation

## MATERIALS AND METHODS

The quantitative analysis of amino acids and dipeptides was performed by elution chromatography on ion-exchange columns with the use of a Beckman/Spinco Unichrom amino acid analyser The same procedure was followed as described by the manufacturers (2) Plasma and serum samples were deproteinized by ultrafiltration before they were applied to the columns of the amino acid analyzer Unless otherwise stated 24 hours urine collections were used The samples were kept frozen until analysis was performed Some toluol was added as preserva

| Subject | Date | Therapy | Administered carnosine | | Urine specimen: 6 hours before loading | | | Urine specimen: 6 hours after loading | | | 1 rease of carn ne excretion | ount on excre ed as percentage of administered carnosine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | (μ moles) | (mg/kg) | Carnosine (μ moles) | Histidine (μ moles) | Creatinine (mg) | Carnosine (μmoles) | Histidine (μ moles) | Creatinine (mg) | (μ moles/ 6 h) | |
| R R ♂ 27 10 1964 | 16 1968 | dd 2 mg Valium® | 610 | 10 | 17 5 | 50 9 | 50 4 | 134 | 57 6 | 55 5 | 116 5 | 19 0 |
| | 27 6 1968 | 2 dd 2 mg Valium® | 327 | 5 | 17 0 | 46 3 | 54 0 | 66 0 | 66 1 | 45 6 | 49 0 | 15 0 |
| | 28 9 1968 | 3 dd 30 mg Luminal® | 318 | 5 | 20 2 | 81 4 | 80 4 | 80 0 | 135 0 | 58 1 | 99 8 | 18 8 |
| E E ♂ 11 1965 | 0 5 1968 | — | 637 | 10 | 4 6 | 3 3 | 60 7 | 39 6 | 32 0 | 56 5 | 35 0 | 5 5 |
| W D ♂ 14 3 1964 | 29 4 1968 | — | 460 | 5 | 25 3 | 12 | 87 0 | 57 3 | 77 0 | 82 1 | 32.0 | 7 0 |

during the 6 hours period before the loading it was calculated that the patient R R excreted after the loading an amount of carnosine which was equivalent to 19 0 15 0 and 18 8% of the administered dosage Perry *et al* (8) performing a similar experiment found that their patients excreted 15* and 8 7* respectively of the administered carnosine in the urine during the first 4 hours period after the loading *Perry et al* (8) detected carnosine in plasma of both their patients after loading with L-carnosine in amounts of 1 0 and 0 3 μmoles carnosine per 100 ml plasma respectively We did not find any carnosine in 3 ml plasma of our patient one hour after loading Since our analyzer could detect amounts of carnosine exceeding about 0 005 μmole it had been concluded that our patient s plasma contained less than 0 17 μmole per 100 ml of plasma

The e loading tests demonstrate that patient R R excreted in the urine a considerably higher amount of the orally administered L carnosine than the control subjects This result was in good agreement with the finding of Perry *et al* (8) They also determined the urinary excretion of carnosine after oral loading with L-carnosine in an unclassified mental defective a mongolism and a healthy adult and found percentages of administered carno ine excreted as high as 4 3 1 3 and 0 4 respectively

*Excretion of carnosine in the urine of patient s relations*

The excretion of carnosine had also been determined once in the urine of relations of patient R R (Table 4) It appeared that both parents of our patient and also one sister of the patient s mother exhibited marked carnosinuria Measurable amounts of carnosine had been detected in the urine of some of our patient s other relations Perry *et al* (8) found no carnosine in the urine of both parents of one of their patients (Case 1) on a regular diet As to the urinary carnosine excretions by relations of our patient it should be noted that the values mentioned in Table 4 repre ent the daily

Table 2 *Daily urinary excretion by patient R R of carnosine anserine histidine 1 methylhistidine β alanine and creatinine under different conditions*

| Urine specimen (date) | Diet | Total µmoles | | | | | Creatinine (mg) |
|---|---|---|---|---|---|---|---|
| | | Carnosine | Anserine | Histidine | 1 Methyl histidine | β Alanine | |
| 4–5 7 1967 | Regular | 218 | — | 292 | 35 | 10 | 183 |
| 19 20 7 1967 | Regular | — | — | — | — | 0 | 223 |
| 9–10 8 1967 | Regular | 168 | 0 | 346 | 60 | — | 193 |
| 22–23 1 1968 | Regular | 200 | — | 355 | 46 | 0 | 259 |
| 23–24 1 1968 | Regular | 295 | 0 | 416 | 70 | — | 296 |
| 27 28 1 1968 | Meat free | 92 | 0 | 348 | — | Trace | 228 |
| 28–29 1 1968 | Meat free | 78 | — | 315 | — | — | 317 |

— = Not determined

24 hours urine was collected 5 times during the regular diet period and twice during the carnosine free diet period The excretions of carnosine anserine histidine 1 methylhistidine and β alanine during the regular diet period and carnosine free diet period were summarized in Table 2 On a regular diet our patient excreted on an average 220 µmoles of carnosine in 24 hours The amount of carnosine excreted decreased to a mean value of 85 µmoles when the patient was taking a meat free diet On a regular diet as well as on a meat free diet no detectable amounts of anserine were found in the patient s urine So it can be stated that a marked carnosinuria existed even on a meat free diet Similar results were obtained by Perry *et al* (8) who reported on a mentally defective patient with carnosinuria This investigator found 170 µmoles of carnosine in 24 hours urine on a regular diet and a mean value of 87 µmoles after a meat free diet period for 72 hours Perry *et al* (8) did not find β alanine and 1 methylhistidine in the urine of their patient on any occasion Our patient excreted only trace amounts of β alanine on a meat free diet During the regular diet period the daily excretion of β alanine was measured 3 times Only one 24 hours urine portion contained a detectable amount of this substance (10 µmoles) Oral loading with 315 µmoles of β-alanine did not result in excretion of β-alanine in urine by our patient, the carnosine excretion remained constant after this loading This suggests that β alanine is readily metabolized So, the increased carnosine excretion cannot be explained in terms of an expanded β-alanine pool resulting in increased carnosine synthesis (11) 1 Methylhistidine a hydrolysis product of anserine, was not excreted by Perry s patient However we found on a regular diet a mean daily excretion of 53 µmoles This discrepancy cannot be explained at present but it is possible that this 1 methylhistidine might be from dietary sources other than anserine (4)

We found no carnosine in our patient s fasting plasma on any occasion Considering the fact that our amino acid analyzer could detect amounts of carnosine exceeding about 0 005 µmole it had been concluded that our patient s plasma (10 ml was used for analysis) contained less than 0 05 µmole of carnosine in 100 ml of fasting plasma on a regular diet

*Oral loading with L-carnosine*

In order to obtain more information about the urinary excretion of carnosine the patient and 2 control subjects (unclassified mental defectives) who showed a measurable carnosinuria were loaded orally with L-carnosine in dosages of 5 to 10 mg per kilogram of body weight during a meat free diet period Urine was collected during a 6 hours period before and a 6 hours period after the loading The results of this experiment have been shown in Table 3 After correction for the carnosine excretion

Table 5 *Serum carnosinase activity of patient R R his parents and three control subjects*

| Subject | Birth date | Age (years) | Carnosinase activity (μmoles of substrate hydrolysed/ml serum/16 h) | |
|---|---|---|---|---|
| | | | Carnosine | Anserine |
| Patient R. R. | 27 10 1964 | 3 8/12 | 0 52 | 0 |
| | | | 1 12 | — |
| Patient's father | 10 10 1936 | 31 | 5 77 | — |
| Patient's mother | 15.2 1940 | 28 | 5 27 | 3 0 |
| Y B | 9 11 11 1955 | 12 7/12 | — | 15 9 |
| R P | 2 1 1959 | 9 7/12 | 16 4 | 11 7 |
| H R | 9 20 3 1960 | 8 2 12 | 16 9 | — |

— Not determined

with a serum-carnosinase deficiency it seemed worthwhile to investigate the influence on both phenomena of an intravenous infusion with freshly prepared donor plasma containing active carnosinase Our patient was given plasma of 2 donors (10 ml/h) during 45 hours Urine specimens were collected in 12 hourly periods from 12 hours before the beginning of the infusion until 27 hours after the end of the infusion Blood samples for determination of the serum-carnosinase activity were taken before the beginning and at the end of the infusion period and furthermore at 27 and 51 hours and 9 days after the infusion had been stopped The results of this experiment have been shown in Table 6 It appeared that the urinary carnosine excretion strongly decreased as a result of the infusion being minimal at the end of the infusion period After that the excretion increased again It also appeared that the serum-carnosinase activity was highest during the period in which the carnosine excretion was minimal After removal of the infusion the serum-carnosinase activity decreased rapidly again This experiment strongly suggests that the carnosinuria observed in our patient was related to the strongly decreased serum-carnosinase activity

Table 6 *Urinary carnosine excretion and serum carnosinase activity before during and after infusion with donor plasma on a meat free diet*

The infusion period began on October 3 1968 at 1200 hours and continued until October 5 1968 at 900 hours

| Date and period of time | (μmoles/mg of creatinine) |
|---|---|
| *Urine specimen* | *Carnosine* |
| 27 28 1 (900 900) | 0 39 |
| 8- 9 1 (900–900) | 0 36 |
| 3 10 (2400–1200) | 0 29 |
| 3 10 (1200–2400) | 0 35 |
| 4 10 (2400–1200) | 0 16 |
| 4 10 (1200–2400) | 0 13 |
| 5 10 (2400–1 00) | 0 09 |
| 5 10 (1200–2400) | 0 13 |
| 6 10 (2400–1200) | 0 20 |
| *Blood sample* | *Carnosinase activity* (μ moles of carnosine hydrolyzed/ml serum/16 h) |
| 23 9 (900) | 2 4 |
| 5 10 (900) | 10 5 |
| 6 10 (1200) | 3 9 |
| 7 10 (1 00) | 3 5 |
| 14 10 (900) | 1 8 |

The carnosinase activity of the donor plasma amounted to 17 3 μ moles of carnosine hydrolyzed in 16 h by 1 ml plasma

## DISCUSSION

Up till now several cases of carnosinuria have been described Bessman & Baldwin (3) demonstrated that 5 patients with juvenile amaurotic idiocy (Spielmeyer Vogt) had a general imidazole amino-aciduria Carnosine as well as anserine excretion had been increased No carnosine was present in the blood of these patients In a following article Tocci & Bessman (15) reported that the patients had an abnormal catabolism of histidine which resulted in d synthesis and excretion of carnosine

Table 4 *Urinary excretion of carnosine by all first and second grade relations of patient R R*

| | Birth date | Relationship | µmoles of carnosine/24 h |
|---|---|---|---|
| J J | 15 2 1940 | Mother of patient | 274 |
| D R | 10 10 1936 | Father of patient | 179 |
| B K | 11 6 1908 | Maternal grandmother | Trace |
| H W | 21 1 1905 | Paternal grandmother | Trace |
| H R | 11 1 1903 | Paternal grandfather | Trace |
| H R | 1 3 1927 | Brother of patient s father | Trace |
| H R | 23 3 1932 | Brother of patient s father | Trace |
| W R | 25 3 1934 | Brother of patient s father | 33 9 |
| A R | 17 3 1928 | Sister of patient s father | 56 3 |
| G R | 16 5 1943 | Sister of patient s father | 37 4 |
| M S | 27 3 1945 | Brother of patient s mother | 42 3 |
| J J | 2 10 1937 | Sister of patient s mother | 222 |
| I J | 18 3 1943 | Sister of patient s mother | 51 2 |
| M J | 17 9 1946 | Sister of patient s mother | 69 0 |

excretion of the dipeptide on a regular diet We were not able to determine the urinary carnosine excretion by relations of patient R R while those persons were taking a meat free diet Block *et al* (4) showed that the excretion of carnosine strongly depended on the diet This author described the carnosine excretion of 5 normal adults and found values ranging from 50 to 500 µmoles depending on the diet From Table 4 it appears that the carnosine excretion of both parents of our patient lie within this range

*Enzymatic activity of carnosinase in serum*

Serum of normal children and adults contains the enzyme carnosinase which catalyzes the hydrolysis of the dipeptides carnosine and anserine into β alanine and either histidine or 1 methylhistidine In order to investigate whether the increased urinary excretion of carnosine by our patient was due to a defect in the metabolism we determined the serum carnosinase activity in our patient and also in his parents and 3 control subjects A similar procedure as described by Perry *et al* (9) was used, with the exception of the amount of serum which was 3 fold in our incubation mixture in order to increase the amount of L carnosine hydrolyzed during the 16 hours period The carnosinase activity (expressed as µmoles of carnosine hydrolyzed in 16 hours per ml of serum) has been shown in Table 5 Perry *et al* (9) found carnosinase activities in serum of their two patients as high as 0 to 0 8 and 0 7 respectively (expressed in the same manner as Table 5) The enzymatic activity in the serum of our patient therefore was in the same order of magnitude Perry *et al* (9) determined the carnosinase activity in serum of 9 healthy children (between 1 and 8 years) and found activities in the range of 6 to 22 3 Our control subjects lie well in this range In 13 adults Perry *et al* (9) found serum-carnosinase activities between 7 7 and 31 2 So it can be stated that both parents of our patient possessed at least a decreased serum carnosinase activity compared to the values given by Perry *et al* (9) The enzymatic experiment mentioned above has also been performed with L anserine nitrate as substrate under similar experimental conditions (Table 5) No anserine was hydrolyzed by the serum of our patient Two control subjects showed enzymatic activity against L anserine in the same order of magnitude compared to L-carnosine Serum of our patient s mother also exhibited decreased activity against anserine

*Urinary carnosine excretion and serum-carnosinase activity before during and after the patient was given an intravenous infusion with freshly prepared donor plasma*

After establishing that the increased urinary carnosine excretion by our patient was coupled

showing a good carnosinase activity we may conclude that the two biochemical symptoms of the syndrome can be corrected

Perry *et al* (8) reported that their two patients had unusually high concentrations of homocarnosine in the cerebrospinal fluid as a result of the carnosinase deficiency and they speculated on a link between the neurological disorder and this phenomenon The concentration of homocarnosine was about 10 times higher than the value reported by Abraham et al (1) Recently however Perry *et al* (10) demonstrated that the concentration of homocarnosine in cerebrospinal fluid of their patients had not been elevated at all considering the normal range for young children Further more they reported that homocarnosine was not hydrolyzed by carnosinase No answer can be given at present to the question whether a relationship exists between the neurological disorder in these patients and the defective cleavage of carnosine

Comparison of the patients described by Perry *et al* (8 9) with the case presented in this article revealed the following common features

1 A progressive neurological disorder with severe mental retardation
   Increased urinary carnosine excretion on a meat free diet
3 Decreased serum-carnosinase activity
4 Electro-encephalographic abnormalities
5 Indications to suppose a genetically determined abnormality


## SUMMARY

A patient with severe mental retardation generalized convulsions and electro-encephalographic abnormalities has been described A marked carnosinuria was observed on a regular diet as well as on a diet which was *essentially* free of carnosine No carnosine was found in plasma of this patient on any occasion The serum-carnosinase activity was extremely diminished compared *to control* subjects Oral loading with [illegible] ed in a much greater excretion of the administered L-carnosine compared to a control subject The consanguineous parents of the patient also excreted large amounts of the dipeptide and had decreased serum-carnosinase activities Infusion with freshly prepared donor plasma containing active carnosinase resulted in a temporary elevation of the enzymic activity in serum and a decrease of carnosine excretion in urine


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and anserine Levenson *et al* (7) investigated 15 Swedish patients with this disease and found that at least some patients had occasionally increased excretion of carnosine Perry *et al* (8) recently described two cases of carnosinuria Two children with a progressive neurological disorder excreted large amounts of carnosine in their urine The carnosinuria remained even when these patients consumed a diet essentially free of carnosine One of the patients had carnosine in fasting plasma even when he consumed a meat free diet Another patient described by the same authors also exhibited carnosinuria but carnosine was not detectable in the fasting plasma after a carnosine free diet period of twelve hours On a regular diet the first mentioned patient of Perry *et al* (8) had 0 65 μmoles of carnosine in 100 ml of fasting plasma and 0 40 μmoles after a meatfree diet period of 72 hours

In a following article Perry *et al* (9) showed that little or no carnosinase activity was present in the sera of their two patients The parents of one patient were first cousins A striking difference between the patients of Bessman & Baldwin (3) and those of Perry *et al* (8) had been noted Whereas the former had a general imidazole aminoaciduria (including increased urinary excretion of carnosine anserine *1*-methylhistidine and histidine) the latter exhibited only carnosinuria

Like Perry s patients our patient had a marked carnosinuria on a regular diet as well as on a meat free diet In contrast to the results obtained by Perry *et al* (8) we did not find detectable amounts of carnosine in plasma of our patient on any occasion This discrepancy might be due to a difference in the renal clearance between Perry s and our patient The latter was calculated from the mean daily carnosine excretion on a regular diet which amounted to 220 μmoles and from the carnosine concentration in fasting plasma being maximally 0 05 μmole in 100 ml The 24 hours renal clearance of carnosine of our patient, aged 36 months was at least 300 ml/min From the values given by Perry *et al* (8) for the carnosine concentration in plasma and for the daily urinary carnosine excretion we calculated the clearance of carnosine of their patient On a regular diet, a meat free diet for 72 hours and a chicken breast diet for 24 hours, the renal clearance of carnosine were found to be 23, 20 and 19 ml/min, respectively The same investigators also described the results of an experiment concerning response of two normal healthy adults to dietary loads of carnosine From these results we calculated the clearance of carnosine and found values as high as 160 and 340 ml/min

It seemed likely that Perry s patient had a strongly decreased clearance of carnosine compared to our patient Extensive studies of the renal function of our patient revealed no abnormalities

So we suggest that the name carnosinemia proposed by Perry *et al* (8) for this disease should be replaced by deficiency of serum carnosinase activity coupled with carnosinuria From our experiments it might be concluded that the dipeptiduria can probably be considered to be a no-threshold type

Our patient possessed only very low serum carnosinase activity The parents of our patient probably had an increased excretion of carnosine Carnosinase activity in their serum appeared to be decreased compared to the values given by Perry *et al* (9) for normal healthy adults It seems very likely that the carnosinuria is the result of a genetically determined deficiency of serum carnosinase activity as proposed by Perry *et al* (9) It is reasonable to accept this hypothesis since the parents of our patient and also at least the parents of one of Perry s patients were consanguineous Furthermore the parents of our patient excreted large amounts of carnosine in urine and had decreased serum carnosinase activities Determination of the serum-carnosinase activity might be useful to detect carriers of the described disease The consanguineous parents of Perry s patient on the contrary did not excrete carnosine in urine From the experiment with intravenously given freshly prepared plasma,

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# THERAPEUTIC STUDIES IN OSTEOPETROSIS

## *Report of 4 Cases*

PETER JOHAN MOE and ARNLAUG SKJÆVELAND

*From the Department of Pediatrics University of Bergen Bergen Norway*

In its typical generalized form osteopetrosis is a rare disease of childhood even beginning in utero as soon as ossification takes place There are however many cases of osteopetrosis which remain symptomless for years The severe form is different clinically and genetically from the milder form which usually manifests itself in adolescence The severe form is inherited as if determined by an autosomal recessive gene the milder form by an autosomal dominant gene (2) The cause of the skeletal abnormality is unknown and many hypotheses have been advanced No dysfunction of the parathyroid gland has been revealed nor any disorder linked to vitamin D metabolism

The osteopetrotic bone is according to Zetterstrom (16) built up of all the types of skeletal tissue which appear during the normal development of bones The mineralized structure is however not removed at a normal rate The primitive mineralized tissue undergoes secondary changes while new formation is taking place instead of being replaced by mature bone marrow tissue This defective development seems to be responsible for the encroachment of the marrow cavity and the resulting extramedullary haematopoiesis in the malignant form Microradiographic studies indicate that there is a very high calcification activity in this abnormal bone tissue (4)

The clinical manifestation of the malignant form usually takes place in the first months of life with enlarged liver and spleen generalized lymphadenopathy haemolytic anaemia thrombocytopenia and immature myeloid cells in peripheral blood There is a high incidence of fractures hydrocephalus and symptoms caused by narrowing of the cranial foramina Loss of vision due to atrophy of the optic nerve and paralysis of the muscle innervated by cranial nerves are frequent symptoms In some case deafness may be present Radiograms show a marked generalized osteosclerosis with a rickets like clubbing of the metaphyses in the long bones

The prognosis is unfavourable especially when haemolytic anaemia and thrombocytopenia are present These infants seldom survive the first year of life Most textbooks state that no effective therapy exists in this disorder During recent years further investigation has been carried out and several forms of treatment have been attempted

The main purpose of this publication is to report a favourable response to prednisone in our last two cases of osteopetrosis The intention of the treatment schedule has been (1) To influence the skeletal changes (2) To prevent cranial nerve lesion especially optic atrophy with blindness (3) To control the haemolytic process and the thrombocytopenia (4) To treat infections

Dent suggested in 1965 (3) that osteopetrosis might be due to a primary biochemical abnormality resulting from an overabsorption of calcium from the diet Calcium studies in one child confirmed a very marked positive calcium balance Dent's results with cellulose

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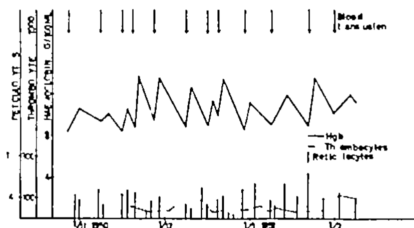



*Fig 1* Case 2 The effect of blood transfusions on the haemoglobin concentration and the reticulocyte and thrombocyte counts

phosphate and prednisone and a report in 1967 (11) on the influence of heparin on urinary calcium excretion in an infant with osteopetrosis stimulated our interest in studying the use of heparin and cellulose phosphate in addition to prednisone in our last two cases

## CASE REPORTS

Three cases of osteopetrosis admitted to the Children's Hospital and a sister of one of them are included in this report The siblings Cases 1 and 2 have been reported previously (15)

*Case 1* Female infant born in 1940 She died at home at the age of 5 months Her parents and two siblings were healthy Case 1 had the same symptoms as her younger brother seizures enlarged liver and spleen and anaemia She was exhumed 10 years after death and the diagnosis osteopetrosis confirmed (15)

*Case 2* Male infant born in 1950 He was admitted to the Children's Hospital at the age of 6 weeks because his mother felt that he might have the same disorder as his sister Pregnancy was uneventful delivery uncomplicated birth weight 4230 g length 52 cm He had seizures from the age of 6 days and enlarged liver and spleen were noticed

On admission he was pale but in good general condition He measured 56 cm (25–50 percentile) and weighed 4720 g (50 percentile) Head circumference was 39 cm the anterior fontanelle was large 4×6 cm Ophthalmoscopic examination revealed pale discs Haemoglobin concentration was 8 1 g per 100 ml white blood cells 24 800 with immature myeloid cells and nucleated red cells in blood smears reticulocyte counts was 3 8 per cent (Fig 1) The concentration of calcium was 10 3 mg per 100 ml and fell to 8 4 mg per 100 ml The serum phosphorus was 2 4–3 7 mg per 100 ml and serum iron 134 μg per 100 ml

Radiographic examination revealed the typical picture of osteopetrosis A marked rosary and swelling of the epiphyses were noticed from the age of about 2 / months Therapy consisted mainly of 17 blood transfusions This had only a transitory effect on the haemoglobin concentration and no definite effect on the number of reticulocytes and thrombocytes (Fig 1)

He suffered from recurrent fever and respiratory tract infections The head circumference increased to 43 5 cm and he died at the age of 5 months of pneumonia in spite of antibiotic therapy

Autopsy showed osteosclerosis of all bones Practically all marrow cavity was filled with calcified chondro osteoid tissue There was an intense extramedullary haematopoiesis subperiosteally in the lymph nodes spleen liver and kidneys

*Case 3* Female infant was born in March 1967 She is the only child of healthy parents Pregnancy was uneventful delivery uncomplicated birth weight 2490 g length 49 cm An enlarged liver and spleen were felt at the age of 2 / months at which age she was admitted to hospital She was then pale but in good general condition She measured 54 5 cm (10 percentile) and weighed 4700 g (50–75 percentile) Head circumference was 39 4 cm (50 percentile) The anterior fontanelle was bulging She had strabismus and pale optic discs but the pupils reacted to light She had generalized lymphadenopathy and hepatosplenomegaly

Her haemoglobin concentration was 9 1 per 100 ml white blood cells 18 400/mm with immature myeloid cells and nucleated red cells in blood smears Reticulocyte counts was 12 0 per cent platelets 70 000/mm Serum calcium was 7 9 g per 100 ml

Roentgenograms showed typical sclerotic bones lack of corticomedullary differentiation and rickets like clubbing of the epiphyses (Fig 2 a)

Erythrocyte survival studies with Cr labelled cells showed normal life span of the patient's erythrocytes in normal blood (K Halvorsen Immunohaemato-

logical Department) Scanning of liver spleen and bone marrow following auto-transfusion of Tc-labelled red cells (with increased fragility) suggested that the liver and spleen were the main destruction areas (L. E. Egnberg Clinical Physiological Laboratory)

No thyrocalcitonin-like activity could be demonstrated by the method of Raetz *et al* (13). This method, however only detects high levels of thyrocalcitonin (12)

*Therapy and course* She received an initial blood transfusion. Prednisone therapy was started a few days after admission in a dose of 10 mg daily. Haemoglobin concentration increased to 11 9 g per 100 ml The number of reticulocytes decreased to 6 per cent and the number of platelets returned to normal (Fig 3)

Prednisone was discontinued in September 1968 because of calcium studies. Therapy had to be resumed after one month owing to a drop in haemoglobin concentration and platelet count, and she has since then been on prednisone The dose was reduced to 7 5 mg every other day in August 1968 A temporary reduction to 5 mg every other day resulted in a marked increase of the size of the spleen and a drop in the haemoglobin concentration and platelet count A similar reduction was attempted in January 1969 with the same result, and she is now on prednisone 10 mg every other day (Fig 3) The serum calcium concentration increased rapidly to normal values 10 2–10 3 mg per 100 ml, following prednisone therapy

In January 1968 she suffered a progression of the optic nerve atrophy and the left eye was found to be amaurotic The right eye still retained some vision It was therefore decided to perform decompression of the right optic canal to prevent further deterioration of the optic nerve The operation was performed in March 1968 The orbital bone was greatly thickened with narrowed marrow spaces Scattered haematopoietic foci were seen

The procedure was uncomplicated and she soon recovered The child (Fig 4) is now in good condition and her mental and statomotoric development seems to be within normal limits Her length at the age of 2 years is 6 cm (just below 2 5 percentile) and her weight 10 5 kg (75 percentile) Her right eye still retains some vision and she walks around without difficulty

Blood values are maintained as shown in fig 3 at satisfactory levels although with slight anaemia and thrombocytopenia

No definite improvement can be seen in the roentgenograms except for the disappearance of the rickets-like process (Fig 2 *b*)

*Case 4* Male infant born in July 1967 He is the only child of healthy parents Pregnancy was uneventful, uncomplicated delivery 2 weeks before term Birth weight 1980 g length 48–49 cm The parents lived in the Cameroons when the patient was born and he received Nivaquin prophylactic against malaria from the age of 2 ½ months He suffered from

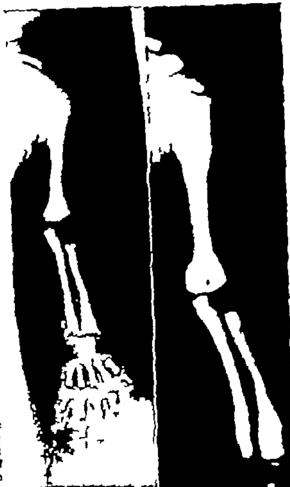


Fig 2 Roentgenogram in Case 3 of the upper extremity at the ages of 2 ½ (*a*) and 21 (*b*) months showing marked sclerosis of the bones and rickets-like changes at the age of 2 ½ months

recurrent fever from the age of 3 ½ months and the parents returned home to Norway for medical care of the infant On admission December 4th 1967 he was 5 months old He was pale and dystrophic weight was 4 7 kg (10 percentile) length 59 cm (2.5 percentile) His head circumference was 41 6 cm (97 5 percentile *referred* to body length) The anterior fontanelle was 2 2 cm slightly bulging He had nystagmus and ophthalmoscopy showed atrophic optic discs The liver and spleen were greatly enlarged and he had generalized lymphadenopathy The haemoglobin concentration was 7 8 g per 100 ml white blood cells 33 000 with immature myeloid cells and nucleated red cells in peripheral blood smears Reticulocyte count was 10 0 per cent and platelet count 36 000/mm (Fig 5) Serum calcium was 8 7–6 9 mg per 100 ml Serum iron was 172 µg per 100 ml TIBC 288 µg per 100 ml and serumbilirubin 0 4 mg per 100 ml No haptoglobin could be demonstrated in the serum

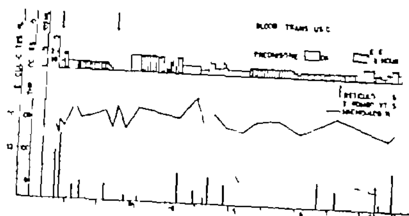


*Fig. 3.* Case 3. The effect of prednisone therapy and blood transfusions on the haemoglobin concentration and the reticulocyte and thrombocyte counts.

Roentgenograms showed the same typical picture as in the previous case.

The results of autotransfusion of Cr-labelled cells revealed a shortened survival time of the patient's red cells. Scanning of liver, spleen and bone marrow following the autotransfusion of Cr-labelled red cells suggested the spleen as the main destruction site. Both studies were performed by K. E. Loenberg. No thyreocalcitonin-like activity could be demonstrated by the method of Rasz *et al.* (13).



*Fig. 4.* Case 3 at the age of 2 years.

*Therapy and course.* He received an initial blood transfusion, antibiotics and prednisone (Actocortin the first days). The infant recovered and there was an increase in haemoglobin concentration and platelet count (Fig. 5). He required however high maintenance doses of prednisone and it was therefore decided to perform a splenectomy. Splenectomy was performed on January 30th 1968. Prednisone was reduced gradually after surgery, but probably too soon. There was a marked fall in haemoglobin concentration and platelet count (Fig. 5) and a tendency towards bleeding. Prednisone treatment was resumed, a dose of 20 mg daily being given at first and gradually reduced later on. He has been on prednisone 15 mg every other day most of the time since July 1968. He has suffered from a moderate haemolytic anaemia all the time. A further decrease in dosage results in increasing anaemia and thrombocytopenia.

He could stand alone at the age of 14 months, but is afraid of walking without help. He seems to have no definite vision, but he reacts to light. Decompression of the optic nerve has so far not been performed. He has a peculiar appearance with a large head, no hair or eyebrows and a short stature. At the age of 18 months his height was only 69 cm (6.4 cm below 2.5 percentile) and his weight was 8 kg (25 percentile). Roentgenograms show an increase in rickets-like changes with no definite improvement of the sclerosis.

## Family Studies

No consanguinity has been found in the three reported families, which are not related in any way. Roentgenographic, haematological and serological studies of the parents (and some of the grandparents) of the four cases have not disclosed any abnormality.

## Calcium Studies

The calcium studies were performed with heparin and cellulose phosphate in addition to prednisone in our last two cases.



Fig. 5. Case 4. The effect of prednisone therapy, blood transfusions and splenectomy on the haemoglobin concentration and the reticulocyte and thrombocyte counts.

In an initial test period both infants were placed on a limited daily calcium intake. The daily calcium intake in Case 3 was about 900 mg and in Case 4 about 750 mg. Urinary and fecal calcium excretions were measured while the infants were without therapy and while they received prednisone, cellulose phosphate or heparin alone or in combinations (Table 1).

The studies had to be interrupted several times owing to difficulties in collecting urine and feces, and only limited studies could be performed due to the need for prednisone in both cases. Studies without prednisone were performed in Case 3 after the patient had been off prednisone for a while and in Case 4 before prednisone therapy was started.

Table 1. *Calcium balance studies in case 3 and 4*

P = Prednisone H = Heparin C = Cellulosephosphate

| Treatment (Daily dosage) | Daily urine calcium | | Daily fecal calcium | | Daily calcium intake |
|---|---|---|---|---|---|
| | No. of days | Mean mg | No. of days | Mean mg | Approx. mg |
| *Case 3* | | | | | |
| None | 3 | 10.3 | | | 900 |
| H 35 mg 2 | 4 | 9.9 | | | 900 |
| P 7.5 mg | 3 | 24.0 | 2 | 430 | 900 |
| H 30 mg 1, P 20 mg | | 23.5 | | | 900 |
| P 7.5 mg, C 8 g | 3 | 22.3 | 3 | 1090 | 900 |
| H 30 mg 1, P 7.5 mg, C 8 g | 1 | 4.9 | 2 | 175 | 900 |
| *Case 4* | | | | | |
| None | 5 | 6.9 | 3 | 280 | 750 |
| P 25 mg | 3 | 10.3 | | | 750 |
| P 10 mg | 3 | 25.3 | 3 | 283 | 750 |
| P 5 mg | 3 | 10.5 | 3 | 136 | 750 |
| H 25 mg, P 5-20 mg | 3 | 16.9 | | | 750 |
| H 30 mg 1, P 5 mg, C 8-10 g | 6 | 10.4 | 5 | 606 | 750 |
| H 30 mg 1, C 8 g | 1 | 5 | 1 | 370 | 750 |

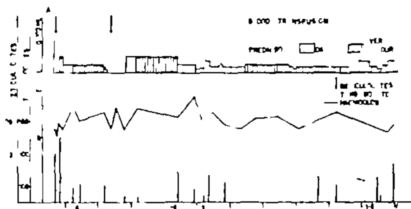


*Fig 3* Case 3 The effect of prednisone therapy and blood transfusions on the haemoglobin concentration and the reticulocyte and thrombocyte counts

Roentgenograms showed the same typical picture as in the previous case

The results of autotransfusion of $^{51}$Cr labelled cells revealed a shortened survival time of the patient's red cells Scanning of liver spleen and bone marrow following the autotransfusion of $^{51}$Cr labelled red cells suggested the spleen as the main destruction site Both studies were performed by K E Leenberg No thyreocalcitonin like activity could be demonstrated by the method of Rasz *et al* (13)



*Fig 4* Case 3 at the age of 2 years

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|---|---|---|---|---|---|
| | No of days | Mean mg | No of days | Mean mg | Approx mg |
| *Case 3* | | | | | |
| None | 3 | 10 3 | | | 900 |
| H 35 mg 2 | 4 | 9 9 | | | 900 |
| P 7.5 mg | 3 | 4 0 | 2 | 430 | 900 |
| H 30 mg 1<br>P 20 mg | 2 | 23 5 | | | 900 |
| P 7 5 mg<br>C 8 g | 3 | 22 3 | 3 | 1090 | 900 |
| H 30 mg 1<br>P 7 5 mg<br>C 8 g | 1 | 4 9 | 2 | 175 | 900 |
| *Case 4* | | | | | |
| None | 5 | 6 9 | 3 | 200 | 750 |
| P 25 mg | 3 | 10 3 | | | 750 |
| P 10 mg | 3 | 5 3 | 3 | 233 | 750 |
| P 5 mg | 3 | 10 5 | 3 | 136 | 750 |
| H 25 mg 2<br>P 5 10 mg | 3 | 16 9 | | | 750 |
| H 30 mg 1<br>P 5 mg<br>C 8 10 g | 6 | 10 4 | 5 | 606 | 750 |
| H 30 mg 1<br>C 8 g | 1 | 5 | 1 | 370 | 750 |

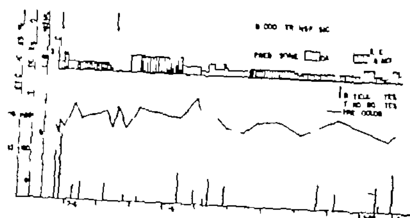


*Fig 3* Case 3 The effect of prednisone therapy and blood transfusions on the haemoglobin concentration and the reticulocyte and thrombocyte counts

Roentgenograms showed the same typical picture as in the previous case

The results of autotransfusion of Cr labelled cells revealed a shortened survival time of the patient's red cells S anning of liver spleen and bone marrow following the autotransfusion of Cr labelled red cells suggested the spleen as the main destruction site Both studies were performed by K E Feenberg No thyreocalcitonin like activity could be demonstrated by the method of Rasz *et al* (13)



*Fig 4* Case 3 at the age of 2 years

*Therapy and course* He received an initial blood transfusion antibiotics and prednisone (Actocortin the first days) The infant recovered and there was an increase in haemoglobin concentration and platelet count (Fig 5) He required however high maintenance doses of prednisone and it was therefore decided to perform a splenectomy Splenectomy was performed on January 30th 1968 Prednisone was reduced gradually after surgery but probably too soon There was a marked fall in haemoglobin concentration and platelet count (Fig 5) and a tendency towards bleeding Prednisone treatment was resumed a dose of 20 mg daily being given at first and gradually reduced later on He has been on prednisone 15 mg every other day most of the time since July 1968 He has suffered from a moderate haemolytic anaemia all the time A further decrease in dosage results in increasing anaemia and thrombocytopenia

He could stand alone at the age of 14 months but is afraid of walking without help He seems to have no definite vision but he reacts to light Decompression of the optic nerve has so far not been performed He has a peculiar appearance with a large head no hair or eyebrows and a short stature At the age of 18 months his height was only 69 cm (6 4 cm below 2 5 percentile) and his weight was 8 kg (25 percentile) Roentgenograms show an increase in rickets like changes with no definite improvement of the sclerosis

### Family Studies

No consanguinity has been found in the three reported families which are not related in any way Roentgenographic haematological and serological studies of the parents (and some of the grandparents) of the four cases have not disclosed any abnormality

### Calcium Studies

The calcium studies were performed with heparin and cellulose phosphate in addition to prednisone in our last two cases

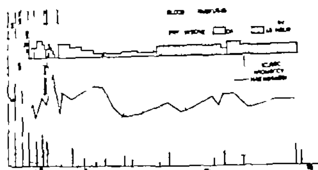


Fig 5 Case 4 The effect of prednisone therapy blood transfusions and splenectomy on the haemoglobin concentration and the reticulocyte and thrombocyte counts

In an initial test period both infants were placed on a limited daily calcium intake The daily calcium intake in Case 3 was about 900 mg and in Case 4 about 750 mg Urinary and fecal calcium excretions were measured while the infants were without therapy and while they received prednisone cellulose phosphate or heparin alone or in combinations (Table 1)

The studies had to be interrupted several times owing to difficulties in collecting urine and feces and only limited studies could be performed due to the need for prednisone in both case Studie without prednisone were performed in Ca e 3 after the patient had been off prednisone for a while and in Case 4 before prednisone therapy was started

Table 1 *Calcium balance studies in case 3 and 4*

P Prednisone H – Heparin C – Cellulosephosphate

| Treatment (Daily dosage) | Daily urine calcium | | Daily fecal calcium | | Daily calcium intake |
|---|---|---|---|---|---|
| | No of days | Mean mg | No of days | Mean mg | Approx mg |
| Case 3 | | | | | |
| None | 3 | 10 3 | | | 900 |
| H 35 mg 2 | 4 | 9 9 | | | 900 |
| P 7 5 mg | 3 | 4 0 | 2 | 430 | 900 |
| H 30 mg 1, P 70 mg | 2 | 23 5 | | | 900 |
| P 7 5 mg, C 8 g | 3 | 22 3 | 3 | 1090 | 900 |
| H 30 mg 1, P 7 5 mg, C 8 g | 1 | 4 9 | 2 | 175 | 900 |
| Case 4 | | | | | |
| None | 5 | 6 9 | 3 | 280 | 750 |
| P 25 mg | 3 | 10 3 | | | 750 |
| P 10 mg | 3 | 25 3 | 3 | 283 | 750 |
| P 5 mg | 3 | 10 5 | 3 | 136 | 750 |
| H 25 mg 2, P 5– 0 mg | 3 | 16 9 | | | 750 |
| H 30 mg 1, P 5 mg, C 8 10 g | 6 | 10 4 | 5 | 606 | 750 |
| H 30 mg 1, C 8 g | 1 | 5 | 1 | 370 | 750 |

Table 1 shows that heparin, 35 mg × 2 daily had no influence on the urine calcium excretion in Case 3

High fecal calcium excretion was found during most of the time the two infants were receiving cellulose phosphate Actually there seems to have been a negative calcium balance on several of those days, while a marked positive calcium balance was found on days without cellulose phosphate (Table 1)

There was a definitely higher urine calcium excretion on the prednisone days compared to the days with no therapy The data in Table 1 permit no conclusion as to the possible influence of prednisone on calcium absorption

A therapeutic trial with all three substances was also attempted Both patients have been on prednisone most of the time since their first admission (Fig 3 and 5) Patient 3 refused to take cellulose phosphate after a short time but she received heparin for about 8 months The dosage had to be adjusted according to coagulation time Prolonged heparin therapy had no demonstrable clinical or roentgenographic effect on the skeletal system nor did it lead to any changes in serum calcium

Case 4 received cellulose phosphate from April 1968 to February 1969 On several occasions 10 g cellulose phosphate was given daily divided into four doses but doses of this size resulted in a drop in calcium concentration to about 6 mg per 100 ml Most of the time the patient therefore received 7 5 daily divided into three doses, resulting in a calcium serum concentration of about 7 mg per 100 ml The administration of cellulose phosphate had no clinical or roentgenographic effect on the skeletal system

## DISCUSSION

The four cases of osteopetrosis described were all similar the manifestations within the first months of life including anaemia thrombocytopenia and hepatosplenomegaly They therefore lend themselves to some degree to the evaluation of therapy even considering the difference in time The two first cases were siblings One of them received no therapy and died at the age of 5 months Case 2 received 12 blood transfusions with only temporary effect on the haemoglobin concentration He also received antibiotic therapy Therapy seems not to have prolonged his life he also died at the age of 5 months

The haematological disturbances and susceptibility to infections present the most urgent problems in the malignant type of osteopetrosis The anemia and thrombocytopenia were controlled in our two last cases with corticosteroid therapy, and infections have not been a problem These cases have now been on prednisone therapy for 21 and 14 months respectively Corticosteroid therapy has probably saved the life of both of them

There are only a few other reported cases of osteopetrosis which have received prednisone most of them with no benefit (1 6 11) Dent (3) reported in 1965 a child who received large daily doses of prednisone for 2 years Steroids could be discontinued following splenectomy

The need for steroids seems to vary in different cases of malignant osteopetrosis Case 3 is doing well on 7 5–10 mg prednisone every other day her anaemia and thrombocytopenia are under control Case 4 had to be kept on large daily prednisone dosage, and it was therefore decided to perform splenectomy He still needs 15 mg prednisone every other day

The anaemia associated with osteopetrosis is not solely if at all due to decrease of the haematopoietic tissue but to an extracorpuscular haemolytic process This has been shown by Sjolin (14) (and concurs with the results of the erythrocyte survival studies performed in Case 3 and 4) It has been suggested that the haemolytic process is due to hypersplenism (5 10 14 16) Splenectomy has been performed in at least eight reported cases of osteopetrosis (1 3 7 10 14) and in six of these with good result in spite of haematopoiesis in the spleen Splenectomy gave a comparatively good result in Case 4 Scanning (page 596) before splenectomy indicated hypersplenism as a cause of the

haemolytic process The anaemia after splenectomy may be due to lack of splenic erythropoesis, or more likely to a haemolytic process in other organs Scanning (page 594) in Case 3 suggested increased erythrocyte destruction in the liver as well as in the spleen This indicates that the haemolytic anaemia in osteopetrosis may be due primarily to a general hyperplasia of the reticuloendothelial system

The thrombocytopenia was corrected by splenectomy in the 4 cases reported by Sjolin This also indicates that hypersplenism exists in osteopetrosis (Sjolin) Discontinuation of prednisone after splenectomy in Case 4 resulted in marked thrombocytopenia and postoperative control of the thrombocytopenia with prednisone proved necessary

It is difficult to explain the mode of action of prednisone on the haemolytic process and thrombocytopenia in osteopetrosis It must in some way be related to the hypersplenism since prednisone could be discontinued in Dent's case and the dosage reduced in Case 4 after splenectomy Similarly reduction of the prednisone dosage to 5 mg every other day in Case 3 resulted in further enlargement of the spleen associated with an increase in the haemolytic process

It has not by any means been proved that the increased mineralization of the skeleton is due to a primary disturbance of the mineral metabolism However the hypocalcemia and rickets like changes in the three studied cases suggest that there may be some disturbances in the calcium metabolism in osteopetrosis

Corticosteroids mobilize calcium from the bones and increase the urinary calcium excretion Prednisone seems to have resulted in normalization of serum calcium in our two treated cases and a disappearance of the rickets like changes in Case 3 within a few months The rickets like changes in Case 4 lately may in that particular case be due to the alternate-day doses of prednisone having failed to influence the rachitic process

Heparin causes an increase in urinary calcium excretion (8) Many patients on long term heparin therapy develop symptoms of osteoporosis This has led to the postulate that heparin may have a stimulating effect on the system responsible for bone resorption in man (3) No definite increase in urinary calcium excretion was demonstrated in Case 3 while she received heparin 35 mg twice daily She received heparin once daily for about 8 months without any demonstrable effect on the skeletal system although this may be because only one dose was given daily It was considered hazardous however to continue heparin therapy twice daily in the face of a persistently prolonged coagulation time

Cellulose phosphate has proved to be effective in preventing gastrointestinal absorption of calcium (3) It has not, however been proved that changes in calcium metabolism and lowered serum calcium have any therapeutic effect on bone changes in osteopetrosis Cellulose phosphate in the doses used resulted in hypocalcemia in Case 4 but had no definite effect on the skeletal system It was considered that increasing the dosage would involve too great a risk

Our studies do not confirm that cellulose phosphate is of any practical use in osteopetrosis This also seems unlikely considering the nature of the disorder

Blindness is a major problem if one succeeds in saving the life of infants with osteopetrosis Two cases of optic nerve decompression have been found in the literature (9) One of them had relatively good vision 3 years after operation The other case only retained the reaction to light which she had before surgery It is important to have decompression performed while vision still remains such as in Case 3 Case 4 has probably no vision and we have hesitated to recommend neurosurgical intervention in this case

## SUMMARY

Four cases of osteopetrosis with manifestations within the first months of life are presented The first two cases were siblings One

of them received no therapy the other 12 blood transfusions and antibiotics Therapy had no influence on the thrombocytopenia and haemolytic process in Case 2 and he developed rachitic like changes and a decrease in serum calcium during hospitalization Both these two siblings died at the age of 5 months The last two cases have been on prednisone therapy for 21 and 14 months respectively Case 3 is now doing well on prednisone 7 5–10 mg every other day Her mental and statomotoric development has so far been normal Optic nerve decompression was performed in March 1968 and the eye on which the operation was performed still retain some vision Case 4 was started on steroid when he was about 3 months older than Case 3 Splenectomy had to be performed due to high prednisone requirements and he still needs prednisone 15 mg every other day He seems unfortunately to be blind and slightly retarded

Heparin seems to be of no practical value in this disorder Cellulose phosphate therapy resulted in impaired calcium absorption The resulting hypocalcemia seems however to have very little if any effect on the underlying disease

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# 5 HYDROXYINDOLEACETIC ACID IN CEREBROSPINAL FLUID OF HYDROCEPHALIC CHILDREN

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Only few biochemical changes in the cerebrospinal fluid (CFS) of patients with hydrocephalus have been reported Normal value of the ionic composition and the acid base balance are described (16) but also changes in the contents of amino acids and the acid monoamine metabolites 5 hydroxyindoleacetic acid (5 HIAA) and homovanillic acid (HVA) (4 5 … 2 19) Similar observations have been reported in experimental hydrocephalus (2 6 7 … 9)

The present work is a more elaborate study on 5 HIAA in CFS of children firstly to compare the levels of 5 HIAA in lumbar CFS or ventricular CFS or both of children with and without hydrocephalus and secondly to correlate the obtained values with such parameters as the clinical course of hydrocephalus the age of the patients and the influence of operative procedures These data might be of diagnostic and prognostic value and of importance in the evaluation of the treatment of hydrocephalus

## MATERIAL AND METHODS

The study is based on measurements of the level of 5 HIAA in CSF obtained at lumbar or ventricular puncture in 191 children (122 boys and 6) girls) in the ages between new born and ten years Twenty nine children had a gestational age below 36 weeks and also a birthweight below 2700 g. They were considered prematurely born The analyses of 5 HIAA were made according to earlier described methods (24 26) The minimum amount of CSF used for the analysis was  0 ml The patients were separated in two main categories The hydrocephalic and the non hydrocephalic group

Children with an enlarged ventricular system in combination with signs of increased intracranial pressure were referred to the hydrocephalic group

Children without this combination of findings were taken to the non hydrocephalic group

Enlargement of the ventricular system was diagnosed either by neuroradiological procedures as lumbar pneumoencephalography and ventriculography or by ventricular puncture with measurement of the thickness of the cerebral mantle usually called ventricular estimation In others echoencephalographies were used and in these cases a ventricular quotient exceeding 0 35 was considered above the normal according to the findings of Sjögren (25)

An abnormally increasing head circumference or an increased fontanel tension or both was taken as evidence for increased intracranial pressure In older children with closed fontanel and sutures the finding of sutural separation was considered as evidence for increased intracranial pressure

1 *The non hydrocephalic" group* comprises children with various diseases and in many cases no specific diagnosis was obtained but they had all in common that they were not hydrocephalic according to the above set criterion This group consisted of 102 patients (70 boys and 32 girls) In fifty three cases of the group there was initially found a head circumference exceeding the mean value for age (21) with 2 standard deviations or more sometimes combined with increased fontanel tension Many of these cases were therefore hospitalized with the suspicion of developing hydrocephalus The diagnoses in the non hydrocephalic group as well as the performed diagnostic procedures are shown in Table 1 None of the patients of this group developed hydrocephalus (observation time 12–48 months)

Ventricular CSF could be obtained in two patients where it was necessary to perform a ventriculography

Table 1 *Diagnoses in the "non hydrocephalic group*

ICP = Intracranial pressure Enc = encephalography Echo-enc = echoencephalography

| Diagnosis | No of cases without signs of increased ICP | Hydrocephalus excluded by | | No of cases with signs of increased ICP | Hydrocephalus excluded by | |
|---|---|---|---|---|---|---|
| | | Enc | Echo-enc | | Enc | Echo-enc |
| Laesio cerebri | 13 | 2 | 8 | 18 | 6 | 12 |
| Subdural effusion | 4 | 2 | 2 | 10 | 8 | 2 |
| Mental retardation | 6 | 1 | 1 | 3 | 3 | — |
| Cerebral atrophy | 4 | 4 | — | 2 | 2 | — |
| Convulsions | 8 | 2 | 4 | 1 | 1 | — |
| Macrocephaly | 2 | — | — | 12 | 1 | 11 |
| Meningitis | 6 | 2 | 2 | 1 | — | 1 |
| Dysplasia | 4 | 1 | 1 | 4 | 2 | 2 |
| Spina bifida cystica | 1 | 1 | — | 2 | 2 | — |
| Cerebral tumor | 1 | 1 | — | — | — | — |
| | 49 | 16 | 18 | 53 | 25 | 28 |

because of lack of gas passage into the ventricles by lumbar route encephalography They showed how ever normal sized ventricles and no further clinical signs of increased intracranial pressure were observed during two years observation They were therefore grouped as non hydrocephalics

Samples of CSF at different ages of the same pa tient were obtained in 19 patients of the non hydro cephalic group (Fig 2 a)

2 *The hydrocephalic group* (89 patients 51 boys and 38 girls) The various diagnoses and the diagnos tic procedures of this group are shown in Table 2 The largest group was hydrocephalus in combination with spina bifida cystica

Eleven of the patients of the hydrocephalic group were not operated upon In the tumour cases removal of the tumour was the initial operative procedure In the other cases a shunting procedure was used

Eight of the eleven not operated cases presented a clinically benign course and it was decided to post pone the operation In six of these eight patients mul tiple 5 HIAA determinations were made which is shown in Fig 2 *b* The three remaining cases which were not operated for hydrocephalus were one child with a subdural effusion and hydrocephalus After removal of the subdural effusion healed his hydro cephalus without further surgical intervention One case had an aqueductal stenosis but died in heart failure before the planned operation of his hydro cephalus could be performed The last case had severe mental disturbances and a head circumference of 69 cm and it was considered that surgery would not ameliorate his symptoms

In the hydrocephalic group of 89 patients it was made 136 analyses of the concentration of 5 HIAA in CSF In 74 patients determination of 5 HIAA was made before an operation for the hydrocephalus The analysis was in 29 (4 prematures) of these cases made in lumbar CSF in 32 cases (3 prematures) in ventricular CSF and in 13 cases (all fullterm) in both these compartments of CSF Fifteen cases (2 prematures) were not examined before an opera

Table 2 *Diagnoses in the hydrocephalic group*

Enc /Vi = Encephalography and/or ventriculography Echo-enc = echoencephalography *VE* = ventricular estimation

| Diagnosis | Operated cases | Hydrocephalus verified by | | Not operated cases | Hydrocephalus verified by | |
|---|---|---|---|---|---|---|
| | | Enc /Vi | VE | | Enc /Vi | Echo-enc |
| Spina bifida cystica | 41 | 29 | 12 | — | — | — |
| Arachnitis | 19 | 19 | — | 3 | 3 | — |
| Cerebral malformations | 5 | 5 | — | 2 | 2 | — |
| Cerebral tumour | 6 | 6 | — | — | — | — |
| Stenosis of aqueduct | 4 | 4 | — | 2 | 2 | — |
| Subdural effusion | 2 | 2 | — | 1 | 1 | — |
| Intoxication | — | — | — | 1 | — | 1 |
| Unknown cause | 1 | 1 | — | 2 | — | 2 |
| | 78 | 66 | 12 | 11 | 8 | 3 |

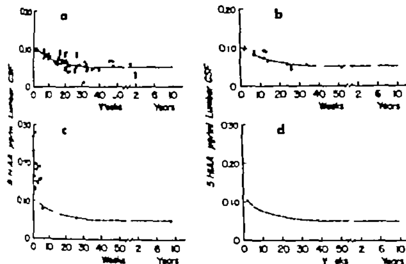



Fig 1 The total series of measurements of 5 HIAA in lumbar CSF pre ratively Each dot represents a single measurement expressed as μg/ml CSF (a) The non hydroceph ic values except those from prematurely born children (82 children and 105 analyses) The supposed normal value line is drawn as a broken line into the other groups b c and d (b) The prematurely born non hydrocephalic" (20) children and 30 analyses) (c) The hydrocephalic group (except prematures) (38 children and 52 analyses) (d) The prematurely born hydrocephalics (4 children and 7 analyses)

... for hydrocephalus and they are presented together with 9 patients where both preoperative and postoperative determinations were done (Table 4)

## RESULTS

The values of 5 HIAA showed a decrease with increasing age (Fig 1 a 2)

As there was a considerable difference in the ... of distribution between the non hydrocephalic" and the hydrocephalic groups we desisted from calculating the mean values of the total groups and compared instead the means of the values from new born up to 12 weeks (the very young group) of the nonhydrocephalic and the hydrocephalic group Before this comparison the values from prematurely born children were extracted from both groups and plotted separately (Fig 1 b d) because we had observed that these children had slightly higher levels than fullterm children The very young non hydrocephalics ($n=$ 30) had a mean value of $0.087 \pm 0.006$ μg/ml S E M and the value from the corresponding hydrocephalic group ($n=22$) was $0.154 \pm$ 0.009 S E M The difference is highly significant with $p < 0.001$ ($t$ test) The mean value from the prematurely born very young non hydrocephalic children ($n=8$) was $0.098 \pm$ 0.007 S E M and the mean values from the corresponding hydrocephalic prematures ($n=$ 4) was $0.197 \pm 0.022$ S E M The difference

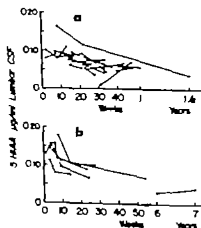



Fig 2 ( ) Nineteen patients in the non hydrocephalic" group with repeated determinations of 5 HIAA at different ages (b) Six patients in the hydrocephalic group which were not operated and subjected to repeated determinations of 5 HIAA at different ages

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| Mental retardation | 6 | 1 | 1 | 3 | 3 | — |
| Cerebral atrophy | 4 | 4 | — | 2 | 2 | — |
| Convulsions | 8 | 2 | 4 | 1 | 1 | — |
| Macrocephaly | 2 | — | — | 12 | 1 | 11 |
| Meningitis | 6 | 2 | 2 | 1 | — | 1 |
| Dysplasia | 4 | 1 | 1 | 4 | 2 | 2 |
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| Diagnosis | Operated cases | Hydrocephalus verified by | | Not operated cases | Hydrocephalus verified by | |
|---|---|---|---|---|---|---|
| | | Enc /Vi | VE | | Enc /Vi | Echo-enc |
| Spina bifida cystica | 41 | 29 | 12 | — | — | — |
| Arachnitis | 19 | 19 | — | 3 | 3 | — |
| Cerebral malformations | 5 | 5 | — | 2 | 2 | — |
| Cerebral tumour | 6 | 6 | — | — | — | — |
| Stenosis of aqueduct | 4 | 4 | — | 2 | 2 | — |
| Subdural effusion | 2 | 2 | — | 1 | 1 | — |
| Intoxication | — | — | — | 1 | — | 1 |
| Unknown cause | 1 | 1 | — | 2 | — | 2 |
| | 78 | 66 | 12 | 11 | 8 | 3 |

HIAA were found in the functioning group

No difference was observed in the levels of 5 HIAA between boys and girls neither in the hydrocephalic nor in the non hydrocephalic group

## DISCUSSION

An obvious difference between the values of 5 HIAA in the CSF from hydrocephalics and non hydrocephalics was immediately observed The latter group was composed of children investigated for various diseases and the series comprised thus no controls in strict sense For the purpose of this investigation however they might be considered relevant A difference between values obtained at ventricular puncture and lumbar puncture was also recognized which has been observed earlier (5) Even if these facts were taken into consideration there was still a great deviation amongst the values This was found partly to depend upon the different ages of the individual patient An inverse correlation was found between the age of the patient and the values of 5 HIAA in lumbar CSF of the non hydrocephalic group At the age of one year their lumbar values were approximating those of adult persons (0 03–0 04 $\mu$g/ml) (20) It was also observed that higher values for age were met with prematurely born children and that these values were relatively higher among the younger premature children One child of the non hydrocephalic group had a very high value at the age of 8 weeks This child was a twin with a birthweight of 1700 g but a gestational age of 40 weeks No other explanation of the high value could be found and two later values were near the normal range for age (Fig 2 *a*) There was no obvious difference between full term children and the older premature (Fig 1 *a b*) A difference in the levels of 5 HIAA in brain tissue has been reported when comparing fetal newborn and older animals but this difference is in the opposite direction with the lowest values of 5 hydroxytryptamine (5 HT) and 5 HIAA in the fetus (13 27) Changes in the turnover of the monoamines in the brain are supposed to be rather closely reflected by changes of the corresponding acids in the CSF (7 9 14 17 18) A successive increase instead of the shown decrease of 5 HIAA in this fluid should therefore be expected if no other factors interfered with the contents of 5 HIAA in the CSF during early life Perhaps the outflow mechanism does not have the capacity of the adult which could be the explanation of the higher levels of 5 HIAA in CSF during the first half year of life However only investigations on the contents of 5 HT and 5 HIAA in the brain of newborn children can give some new evidence on this point

The difference between the 5 HIAA values of the non hydrocephalics and the hydrocephalics was found to be most marked in the very young age group A certain reservation with respect to this statement has to be done because of the relatively few values of 5 HIAA in the hydrocephalic group of 4 to 12 months are Because of the difficulty of comparing these in many respect disparate groups the line of the non hydrocephalic group was drawn and the values of the other group were compared with this line (Fig 1) Studying the lumbar values of the hydrocephalic group it is obvious that most of the hydrocephalic values are well above this line Fifteen values are however below the value of 0 1 $\mu$g/ml and it might be of interest to consider these cases in detail Eight of these values were later determinations from 5 children which were not operated and when these lower values were obtained the signs of increased intracranial pressure had also vanished (cf Fig 2 *b*) Two values were from children with non communicating hydrocephalus The importance of recognizing the values from cases with non communicating hydrocephalus will be discussed later on Two values were from older children operated upon at the ages of 7 and 9 years They had both the history of rapidly increasing head circumference during the first years of life but the last years the situation had

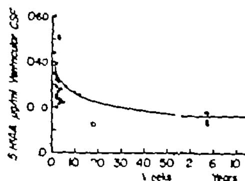



*Fig 3* The total series of measurements of 5 HIAA in ventricular CSF of hydrocephalic children preoperatively (45 children and 48 analyses) Each dot represents a single measurement expressed in µg/ml CSF The circles are two values from non-hydrocephalic children

between these values is also highly significant with $p<0.001$ (*t* test) The mean value for ventricular CSF in the very young hydrocephalic group ($n=26$) was $0.315 \pm 0.022$ S.E.M The two values of ventricular CSF in non-hydrocephalic children of 0.13 and 0.18 at the ages of 18 and 12 weeks respectively are plotted separately in Fig 3

Lumbar and ventricular CSF were taken preoperatively at the same time in 13 cases of the hydrocephalic group (Table 3) In three of these cases there was no passage of air into the ventricles by lumbar pneumo-encephalography or passage of air to the basal cisterns at ventriculography These three cases might thus be comparable with so called non-communicating hydrocephalus The ventriculo-lumbar ratios of the non-communicating group exceeded the mean ratio of the communicating group with +3 S.D (Table 3) In 24 cases the 5 HIAA values were determined after surgery These cases were divided in 12 functioning and 12 malfunctioning shunts (Table 4 *a* *b*) Lower values of ventricular 5

Table 3 *Comparison of ventricular (V) and lumbar (L) values of 5 HIAA with particular reference to air passage to and from the ventricles*

Ten cases had communicating hydrocephalus (C) and 3 cases had an impaired passage— non-communicating (NC) The difference between ventricular and lumbar concentration of 5 HIAA is significant with $p<0.01$ using the difference method The mean value of the ventriculo-lumbar (V/L) ratio in the communicating group is $1.5 \pm 0.56$ S.D

| | 5 HIAA µg/ml | | V L ratio | |
|---|---|---|---|---|
| Age | V | L | C | NC |
| 3 weeks | 0.23 | 0.17 | 1.4 | |
| 3 weeks | 0.50 | 0.15 | | 3.5 |
| 4 weeks | 0.28 | 0.16 | 1.8 | |
| 5 weeks | 0.15 | 0.16 | 1.0 | |
| 9 weeks | 0.29 | 0.1[illegible] | [illegible]3 | |
| 10 weeks | 0.26 | 0.01 | | [illegible]6.0 |
| 12 weeks | 0.26 | 0.18 | 1.5 | |
| 26 weeks | 0.11 | 0.05 | 2.4 | |
| 41 weeks | 0.21 | 0.06 | | 3.4 |
| 1 year | 0.13 | 0.18 | 0.7 | |
| 5 years | 0.15 | 0.16 | 0.9 | |
| 7 years | 0.06 | 0.04 | 1.5 | |
| 9 years | 0.07 | 0.05 | 1.5 | |

Table 4 *Comparison between the values of 5 HIAA in lumbar (L) and ventricular (V) CSF of 24 children before and after operation*

12 functioning and 12 malfunctioning shunts The value in brackets in table b is obtained after 24 hours of ventricular drainage

| Before operation | | | After operation | | |
|---|---|---|---|---|---|
| Age | L | V | Age | L | V |
| *a Functioning shunt* | | | | | |
| 3 weeks | 0.19 | — | 17 weeks | 0.03 | — |
| 3 weeks | 0.13 | — | 9 week | 0.03 | 0.17 |
| 3 weeks | 0.15 | 0.50 | 9 weeks | 0.07 | 0.19 |
| 3 weeks | — | — | 5 weeks | 0.11 | — |
| 8 weeks | — | 0.50 | 5 years | — | 0.1 |
| 19 weeks | 0.05 | — | 6 weeks | 0.06 | — |
| 7 weeks | — | — | 20 weeks | 0.0 | — |
| 17 week | — | — | 43 weeks | 0.07 | — |
| 47 weeks | — | — | 46 weeks | 0.05 | — |
| 1 year | — | — | 1 year | 0.0[illegible] | 0.14 |
| 1 year | — | — | 5 years | 0.03 | 0.05 |
| 6 weeks | — | — | 7 years | 0.07 | — |
| *b Malfunctioning shunt* | | | | | |
| Before operation | | | At revision operation | | |
| Age | L | V | Age | L | V |
| [illegible] weeks | — | 0.29 | 51 weeks | — | 0.[illegible]0 |
| 4 weeks | 0.16 | 0.28 | 17 week | — | 0.37 |
| 4 weeks | — | — | 6 weeks | 0.17 | — |
| 6 weeks | — | 0.34 | 13 weeks | — | 0.[illegible]0 |
| 6 weeks | — | — | 10 weeks | — | 0.22 |
| 2 weeks | — | — | 30 week | — | 0.[illegible]0 |
| [illegible]3 weeks | — | 0.37 | 3 years | — | 0.71 (0.16) |
| 16 weeks | — | — | [illegible]7 weeks | — | 0.15 |
| 3 weeks | — | — | 34 weeks | — | 0.14 |
| 29 weeks | — | — | 35 weeks | — | 0.33 |
| 4 weeks | — | — | 1 year | — | 0.2[illegible] |
| 5 weeks | — | — | [illegible] years | — | 0.[illegible]0 |

— = not done

HIAA were found in the functioning group

No difference was observed in the levels of 5 HIAA between boys and girls neither in the hydrocephalic nor in the non hydrocephalic group

## DISCUSSION

An obvious difference between the values of 5 HIAA in th CSF from hydrocephalics and non hydrocephalics was immediately observed The latter group was composed of children mve treated for various diseases and the series comprised thus no controls in strict sen e For the purpose of this investigation however they might be considered relevant A difference betwe n values obtained at ventricular puncture and lumbar puncture was also recognized which has been observed earlier (5) Even if these facts were taken in consideration there was still a great deviation mon t the values This was found partly to depend upon the different ages of the individual patient An inverse correlation was found between the age of the patient and the values of 5 HIAA in lumbar CSF of the non hydrocephalic group At the age of one year their lumbar values were approximating those of adult persons (0 03–0 04 $\mu$g/ml) (20) It was also ob erved that higher values for age were met with prematurely born children and that these values were relatively higher among the younger premature children One child of the non hydrocephalic group had a very high value at the age of 8 weeks This child was a twin with a birthweight of 1700 g but a gestational age of 40 weeks No other explanation of the high value could be found and two later values were near the normal range for age (Fig 2 *a*) There was no obvious difference between full term children and the older prematures (Fig 1 *a b*) A difference in the levels of 5 HIAA in brain tissue has been reported when comparing fetal newborn and older animals but this difference is in the opposite direction with the lowest values of 5 hydroxy tryptamine (5 HT) and 5 HIAA in the fetus (13 27) Changes in the turnover of the monoamine in the brain are supposed to be rather closely reflected by changes of the corresponding acids in the CSF (7 9 14 17 18) A succe sive increase instead of the shown decrea e of 5 HIAA in this fluid should therefore be expected if no other factors interfered with the contents of 5 HIAA in the CSF during early life Perhaps the outflow mechanism does not have the capacity of the adult which could be the explanation of the higher levels of 5 HIAA in CSF during the first half year of life However only investigations on the contents of 5 HT and 5 HIAA in the brain of newborn children can give some new evidence on this point

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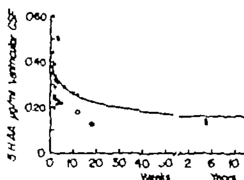



*Fig 3* The total series of measurements of 5 HIAA in ventricular CSF of hydrocephalic children pre operatively (45 children and 48 analyses) Each dot represents a single measurement expressed as µg ml CSF The circles are two values from "non hydrocephalic" children

between these values is also highly significant with $p<0.001$ ($t$ test) The mean value for ventricular CSF in the very young hydrocephalic group ($n=26$) was $0.315 \pm 0.022$ S E M The two values of ventricular CSF in non hydrocephalic children of 0.13 and 0.18 at the ages of 18 and 12 weeks respectively are plotted separately in Fig 3

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| | 5 HIAA µg/ml | | V/L ratio | |
|---|---|---|---|---|
| Age | V | L | C | NC |
| 3 weeks | 0.23 | 0.17 | 1.4 | |
| 3 weeks | 0.50 | 0.15 | | 3.5 |
| 4 weeks | 0.28 | 0.16 | 1.8 | |
| 5 weeks | 0.15 | 0.16 | 1.0 | |
| 9 weeks | 0.29 | 0.12 | 2.3 | |
| 10 weeks | 0.26 | 0.01 | | 26.0 |
| 12 weeks | 0.26 | 0.18 | 1.5 | |
| 26 weeks | 0.11 | 0.05 | 2.4 | |
| 41 weeks | 0.21 | 0.06 | | 3.4 |
| 1 year | 0.13 | 0.18 | 0.7 | |
| 5 years | 0.15 | 0.16 | 0.9 | |
| 7 years | 0.06 | 0.04 | 1.5 | |
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Table 4 *Comparison between the values of 5 HIAA in lumbar (L) and ventricular (V) CSF of 24 children before and after operation*

12 functioning and 12 malfunctioning shunts The value in brackets in table b is obtained after 24 hours of ventricular drainage

| Before operation | | | After operation | | |
|---|---|---|---|---|---|
| Age | L | V | Age | L | V |
| *a Functioning shunt* | | | | | |
| 3 weeks | 0.19 | — | 17 weeks | 0.03 | — |
| 3 weeks | 0.13 | — | 9 weeks | 0.07 | 0.17 |
| 3 weeks | 0.15 | 0.50 | 8 weeks | 0.07 | 0.19 |
| 3 weeks | — | — | 5 weeks | 0.11 | — |
| 8 weeks | — | 0.50 | 5 years | — | 0.12 |
| 18 weeks | 0.05 | — | 26 weeks | 0.06 | — |
| 7 weeks | — | — | 20 weeks | 0.07 | — |
| 17 weeks | — | — | 43 weeks | 0.07 | — |
| 42 weeks | — | — | 46 weeks | 0.03 | — |
| 1 year | — | — | 1 year | 0.05 | 0.14 |
| 1 year | — | — | 5 years | 0.03 | 0.03 |
| 26 weeks | — | — | 7 years | 0.07 | — |
| *b Malfunctioning shunt* | | | | | |
| Before operation | | | At revision operation | | |
| Age | L | V | Age | L | V |
| 2 weeks | — | 0.29 | 51 weeks | — | 0.20 |
| 4 weeks | 0.16 | 0.28 | 17 weeks | — | 0.37 |
| 4 weeks | — | — | 6 weeks | 0.12 | — |
| 6 weeks | — | 0.34 | 13 weeks | — | 0.20 |
| 6 weeks | — | — | 10 weeks | — | 0.22 |
| 2 weeks | — | — | 20 weeks | — | 0.30 |
| 23 weeks | — | 0.37 | 3 years | — | 0.71 (0.16) |
| 16 weeks | — | — | 27 weeks | — | 0.15 |
| 3 weeks | — | — | 34 weeks | — | 0.14 |
| 29 weeks | — | — | 38 weeks | — | 0.33 |
| 4 weeks | — | — | 1 year | — | 0.22 |
| 5 weeks | — | — | 2 years | — | 0.[illegible]0 |

— = not done

the hydrocephalic group (Table 3) In three of these cases there was no passage of air into the ventricles by lumbar pneumo encephalography or passage of air to the basal cisterns at ventriculography These three cases might thus be comparable with so called non communicating hydrocephalus The ventriculo-lumbar ratios of the non communicating group exceeded the mean ratio of the communicating group with $+3$ S D (Table 3) In 24 cases the 5 HIAA values were determined after surgery These cases were divided in 12 functioning and 12 malfunctioning shunts (Table 4 *a b*) Lower values of ventricular 5

between the hydrocephalic children of the same age group could probably depend upon the severity of hydrocephalus or upon different cause of the disease or both In this investigation no attempt was made to correlate the widening of the ventricles and the intracranial pressure with the values of 5 HIAA

Summarizing the findings from this investigation it seems probable that a decreased elimination from CSF is the main cause of the increase of 5 HIAA in CSF of hydrocephalus Other possibilities could however be discussed An increase in the synthesis of 5 HT in the brain could be another explanation of the increased values of the metabolite in CSF There are however data suggesting that 5 HT synthesis is not changed in animals with experimental hydrocephalus (9) Data from experiments with intraventricular injections of 5 HIAA to normal and hydrocephalic dogs speak in favour of the hypothesis of a decreased elimination of 5 HIAA from CSF in hydrocephalus (8) Moreover when these dogs were pretreated with probenecid—a drug not only reducing the renal excretion of 5 HIAA (15) but also decreasing the outflow of the acid from the brain and CSF (14 17 22 28) —there was no further increase of 5 HIAA in CSF This might be explained by no remaining outflow mechanism which the probenecid could further impair


## SUMMARY

There was a significant increase in the level of 5 HIAA in lumbar CSF of children with hydrocephalus compared with non hydrocephalic children In non hydrocephalic children the values of 5 HIAA were found to be highest in the newborn successively decreasing to the levels of adult persons at the age of about one year Prematurely born children seemed to have relatively higher levels of 5 HIAA in CSF compared with full term children Adult persons were not included in the study

The ventriculo-lumbar ratio in different types of hydrocephalus was calculated A diminished level of 5 HIAA was measured after surgery with a functioning shunt All the findings support the theory that the cause of the increased level of 5 HIAA is a decreased elimination of the acid metabolite from CSF Determination of 5 HIAA in CSF might be a simple and useful diagnostic complement in various neurological diseases where the suspicion of hydrocephalus has arisen



## ACKNOWLEDGEMENT

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stabilized and no absolute evidence of increased intracranial pressure was at hand Surgery was here performed in an attempt to relieve *their psychic symptoms* The three remaining low values were found in three patients (18 26 and 37 weeks old) where it was not quite sure if their hydrocephalic state was progressive or not They were subjected to surgery more or less in order to prevent an eventual progress of their hydrocephalus These cases indicate that determination of 5 HIAA might be of value in the decision of surgery in hydrocephalus

In order to get a useful method when diagnosing hydrocephalus it was necessary to study the lumbar values of 5 HIAA Earlier investigations on 5 HIAA in CSF of hydrocephalics have mostly dealt with ventricular CSF (4 5 12) This has limited the value of the method as it is impossible to get a series of normal values on ventricular CSF in humans apart from incidental observations The group of cases where ventriculography is necessary is comparatively small but it actualizes the importance of knowing the ratio between the 5 HIAA values of different parts of the cerebro-*spinal fluid space In experiments on animals* it has been shown that there is a fall of concentration from lateral ventricular to cisternal CSF (2 9 17) and also from cisternal to lumbar CSF (10) This has been explained by the assumption that the main bulk of 5 HIAA enters CSF in the lateral and third ventricles of *the brain and* that the elimination of this as well as other organic acids mainly takes place in the caudal part of the fourth ventricle (11 14 17 23) It was therefore of interest to study the ventriculo-lumbar ratio in our children It might perhaps be possible to calculate the value of 5 HIAA in ventricular CSF by knowing *the lumbar* value of the acid However this presumes a free communication between ventricular and lumbar CSF which is not always the case The hydrocephalic patients were therefore separated in two groups the cases where air entered the lateral ventricles by lumbar encephalography (comparable with so called communicating hydrocephalus) and the cases where air did not pass out of the ventricles at ventriculography (a condition resembling non-communicating hydrocephalus) When comparing these two groups it was *found that the ventricular levels of 5 HIAA* were markedly higher than the lumbar values in the non communicating cases On the other hand there was a more uniform level of 5-HIAA in the communicating type of hydrocephalus (Table 3) The observation of a lower ventriculo lumbar ratio in the communicating *type of hydrocephalus contrasting* to a higher ratio in non communicating hydrocephalus speaks in favour of 5 HIAA mainly entering the CSF in the lateral ventricles and leaving CSF distally to the aqueduct

5 HIAA was measured in CSF postoperatively *in 12 cases where the patients had a functioning* shunt A diminished level of 5 HIAA was observed in most of these cases (Table 4 *a*) The 5 HIAA level was also measured in 12 other cases at revision of a malfunctioning shunt and the ventricular values of these cases were mostly equal with the values of hydrocephalic patients preoperatively (Table 4 *b*) This observation has sometimes been used as a test of shunt performance If the observed decrease of 5 HIAA when the shunt is functioning depends on a pure drainage of 5 HIAA through the shunt system to the blood, or on a better physiological elimination with a normalized *intracranial pressure*, has to be discussed against data indicating that less than 35 % of the CSF passes through the shunt (3) We do not think that the drainage of CSF through the shunt is the only explanation of a decrease of 5 HIAA after the *operation* It seems more plausible that a decrease of the intracranial pressure is the main factor restituting the conditions of elimination of 5 HIAA and thus decreasing the values In one case with a malfunctioning shunt there was a significant drop in the 5 HIAA value after ventricular *drainage* for 24 hours (Table 4 *b*) The observed difference in the levels of 5 HIAA

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# DOWN'S SYNDROME TRANSMITTED THROUGH MATERNAL MOSAICISM

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Following the discovery of $D/G_1$ and $G/G_1$ translocations in Down's syndrome it was supposed that most instances of familial occurrence of the syndrome were caused by transmission through balanced translocation carriers However subsequent studies of a large number of families with more than one affected member including many families with affected sibs have disclosed that only a fraction of the familial cases could be accounted for by inheritance from translocation carriers (8 11 14 17 20 26) Mosaic of normal and G trisomic cells in one of the parents seems to be a rare cause of Down's syndrome in sibs and only one such family has been reported (18)

The purpose of this paper is to describe another family with two affected sibs in whom regular $G_1$ trisomy was inherited from a phenotypically normal mother with normal/$G_1$ trisomy mosaicism and in addition to report a unique case of inherited D/G translocation trisomy in which the carrier mother had a mosaic of normal and balanced $D/G_1$ translocation cells

## CASE HISTORIES

*Case 1* This mother was number 4 among 4 siblings Her mother was 39 years old and her father 45 years old when she was born There was no known case of Down's syndrome in either her or her husband's family She finished elementary school with average grades and before she married worked as a nurses aid in a hospital She had had no abortions At the age of [illegible] years she gave birth to a boy with typical features of Down's syndrome Her next pregnancy ended 18 months later with the birth of another boy who also had typical manifestations of Down's syndrome Both pregnancies were uneventful and there was no history of radiation exposure drug ingestion or viral infection She had no further children

At the time of examination she was 25 years old Her height was 165 cm and body build normal She displayed no stigma of Down's syndrome (Fig 1) Based on clinical assessment, school and social achievement her intelligence was considered normal

Chromosome analysis was carried out on three different occasions in cultured lymphocytes from peripheral blood A total of 166 metaphases were examined Eight contained 47 chromosomes with one extra chromosome in the G group Of the remaining 158 cells there were 156 with a normal female karyotype Both children had regular G trisomy The father was not available for examination

*Case 2* This mother was the second child of healthy parents (Fig 2) There had been no case of Down's syndrome in her family At the time of her birth her mother was 23 years old and her father 30 years There was no history of radiation exposure She had no abortions and her first pregnancy ended with the birth of a normal boy Two years later at the age of 32 years she gave birth to a girl with typical features of Down's syndrome The pregnancy had been uneventful and there was no history of drug ingestion or viral infection

Chromosome analyses of cultured lymphocytes from the child revealed a modal number of 46 chromosomes including 4 G chromosomes 5 D chromosomes and a $D/G$ translocation chromosome Chromosomal analysis of the mother was performed on two different occasions A total of 82 cells were examined Seventy metaphases contained 46 chromosomes with a normal female karyotype Ten cells had 45 chromosomes with 3 G group chromosomes 5 in the D group and a $D/G_1$ translocation chromosome similar to that observed in the child with Down's syndrome Chromosome analysis was also carried out in the child's father and all available members of the

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*Key words* 5 HIAA cerebrospinal fluid hydrocephalus

Table 1. Review of reported cases of Down's syndrome transmitted through mosaic parent

| Reference no. | Mosaic parent and age | Phenotype | Mosaic type | Percentage abnormal cells | | Offspring | | | Parental age at birth of mosaic parent |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Down's syndrome | | Normal | |
| | | | | Blood | Skin | Number | Karyotype | no. | |
| 1 | Mother 28 | Features of Down's syndr. I.Q. 60 | Normal/G trisomy | 14 | | 1 | G trisomy | 0 | Mother's mother 40, Mother's father 42 |
| 4 | Mother 25 | Normal | Normal/G trisomy | .9 | 21 | 1 | G trisomy | 0 | Mother's mother 27, Mother's father 32 |
| 18 19 | Mother 19 | Normal | Normal/G trisomy | 27 | 75 | 3 | G trisomy | 0 | Mother's mother 39 |
| 22 | Mother 20 | Features of Down's syndr. | Normal/G trisomy | .0 | | 1 | G trisomy | | |
| 23 | Mother 32 | Normal | Normal/G trisomy | 10 | | 1 | G trisomy | 1 | |
| 5 | Mother 17 | Normal | Normal/G trisomy | 16 | 18 | 1 | G trisomy | | Mother's mother 39 |
| Present case 1 | Mother 22 | Normal | Normal/G trisomy | 5 | | 2 | G trisomy | 0 | Mother's mother 39, Mother's father 45 |
| 24 | Mother | Normal | Normal/G/G trisomy | 0 | 22 | 3 | G/G trisomy | 1 | |
| 4 | Father 26 | Normal | Normal/D/G trisomy | .0 | | 1 | D/G trisomy | 1 | Father's mother 24, Father's father 34 |
| Present case 2 | Mother 32 | Normal | Normal/D/G carrier | 13 | 1 | | D/G trisomy | 1 | Mother's mother 23, Mother's father 30 |



*Fig 1* Mother with normal/G trisomy mosaic and her two sons with regular $G_n$ trisomy and Down's syndrome

mother's family They all had normal karyotype (Fig 2)

Blood grouping and examination of *Gm Hp Gc* and Pi types in the mother and her relatives did not reveal any inconsistencies suggesting chimerism in the mother

## DISCUSSION

Including the present family a total of 7 families have been reported in which maternal mosaicism of normal/G trisomy type has given rise to regular G trisomy and Down's syndrome in the offspring (Table 1) In one of these families the mother gave birth to three consecutive children with Down's syndrome (18 19), whereas the other previously reported families had only one affected child Five of the mothers were considered to be phenotypically normal (4 18 23 25) while the remaining two displayed some features of Down's syndrome (1 22) The clinical manifestations in mosaic cases of Down's syndrome have varied considerably Although there seems to be a correlation between the mental development and the proportion of trisomic cells (16) such correlation is not evident when the extent of somatic anomalies is considered (2 5) However there is a tendency to more pronounced somatic manifestations in cases with higher proportion of trisomic cells (2) and it will be noted that the phenotypical normal, or subnormal mosaic women who have given birth to regular G trisomic children had less than 30 per cent trisomic cells in lymphocyte cultures (Table 1) In cases where the proportion of euploid to aneuploid cells in leucocyte and fibroblast cultures has been compared it has been noted that the trisomic cells occur about twice as frequently in the fibroblasts as in the leucocytes (13) The number of trisomic cells in the present mosaic mother was rather low but trisomic cells were consistently found in three separate cultures and trisomic cells in similar or even lower proportion have been found in leucocyte cultures from patients with typical features of Down's syndrome (3 9 15)

Inheritance of Down's syndrome through a mosaic parent is probably a rare event since cytogenetic studies of relatively large series of parents with children with regular G trisomy have failed to disclose such mosaicism (7 9 12) Mosaicism normal/G trisomy might arise from a normal zygote containing 46 chromosomes or from an abnormal zygote containing 47 chromosomes with G trisomy It has been noted that the age of mothers at birth of children with mosaic Down's syndrome has tended

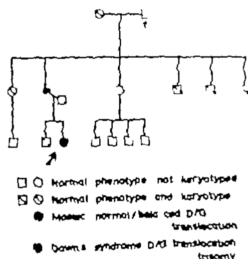



Fig 2 Pedigree showing transmission of $D/G_n$ translocation through mosaic mother

with D/G 1 translocation Down s syndrome Assuming random segregation these three types of gametes should be produced with equal frequency However owing to some pre zygotic selection the chance for the unbalanced gamete to be fertilized is probably nearer 10 per cent than the theoretical 33 per cent (10) Therefore provided an equal gonadal involvement the woman with the mosaic normal/G trisomy has more than twice the risk of the women with normal/balanced D/G 1 transloca tion mo aic of having a child with Down s syn drome


## SUMMARY

Two families are reported in which Down s syndrome was transmitted through phenotypically normal mosaic mother In the first family the mother had normal/G trisomy mosaic and on two successive pregnancies at the age of 22 and 24 years she gave birth to two ds with regular G 1 trisomy and Down s syndrome In the other family the mother had a mo aic of normal and balanced D/G 1 translocation cells and at the age of 32 years she gave birth to a child with D/G 1 translocation trisomy and Down s syndrome

Mosaic mothers carry a considerable risk of having children with Down s syndrome Exact prediction of the risk cannot be given since the extent of gonadal involvement in the mosaicism is unknown


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to deviate towards the higher maternal age group (14) This finding indicates that an age dependent factor may be involved in the genesis of such mosaics, and it is therefore probable that the majority of mosaics originate from a primarily trisomic zygote Information on mosaic mothers is still scanty but the available data show a tendency for these mothers to be rather young when giving birth to their affected child whereas the mother s mothers tend to be at the end of their reproductive period when they gave birth to their mosaic daughters (Table 1)

D/G 1 translocation trisomy inherited from a parent with mosaicism of normal and balanced D/G 1 translocation cells has, to the best of the author s knowledge not been reported previously A somewhat similar condition was described in the father of a girl with D/G 1 translocation trisomy (21) Karyotyping of the father revealed about 10 per cent of cells with a balanced D/G 1 translocation and another 10 per cent normal cells whereas the remaining cells showed an array of different abnormal chromosome complements D/G 1 translocation trisomy was present in the majority of analysed cells from the child but in addition her karyotype also contained a multiplicity of *other chromosomal abnormalities*

The origin of the D/G 1 translocation heterozygosis in the present mother could not be determined with certainty since her father was deceased However the results of chromosome analysis of available family members suggest that the translocation had arisen de novo (Fig 2) The mosaicism might be a result of a post zygotic event and in that case either originated from a normal or a balanced D/G 1 zygote Alternatively the mosaic pattern might be explained as chimerism derived from double fertilization or an absorbed twin However examination of blood types and other genetic markers in the mother and family members did not reveal any inconsistencies suggestive of chimerism

If a parent has a mosaic karyotype in leucocyte or skin tissue cultures it is most probable that the same mosaic exists in gonadal tissue However the ratio of normal to abnormal cells found in other tissues is by no means representative for the condition in the gonads Normal meiotic division of the euploid germ cells with 46 chromosomes will produce gametes with the normal haploid number of 23 The aneuploid germ cells with 47 chromosomes and G 1-trisomy will produce two types of gametes one type with the normal haploid number and the other with 24 chromosomes including one extra chromosome No 21 This *demonstrates secondary or inevitable non* disjunction In women with Down s syndrome and regular G 1-trisomy the theoretically expected number of balanced and unbalanced gametes will be equal So far a total of 15 females with Down s syndrome are known to have born a total of 16 children (6 13) Five children had Down s syndrome Three were stillborn and among these was a pair of twins with definite Down s syndrome Two of the children were mentally retarded without Down s syndrome, and six were normal Within the limits of the small number the occurrence of six births of children with Down s syndrome in 15 pregnancies might be considered consistent with the theoretical expectation

*At present it is not practicable to examine* gonadal involvement in the mosaicism That mothers with normal/G 1 trisomy mosaic carry a considerable risk of having children with Down s syndrome is evident from the findings in the present family and that reported by Smith and co workers (18 19) but exact prediction of the risk cannot be given in the individual case The circumstances concerning assessment of gonadal involvment are similar in parents with mosaic of normal and balanced D/G 1 translocation cells For practical purposes one might consider three types of gametes derived from the germ cells containing the balanced D/G 1 translocation the normal haploid gamete the balanced D/G 1 translocation gamete and the unbalanced D/G 1 translocation gamete Fertilization of the latter gamete by a normal gamete will produce a child

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CASE REPORT

# HEMORRHAGIC MUCOSAL NECROSIS OF THE GASTROINTESTINAL TRACT IN THE NEWBORN

## *Two Cases with Intestinal Perforation Without Vascular Occlusion*

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Hemorrhagic mucosal necrosis of the gastrointestinal tract without vascular occlusion has long been known from experimental shock studies in animals (10) but the condition was first described in humans in 1954 by Wilson & Qualheim (21) under the term hemorrhagic enterocolitis This term is now rightfully seldom used as this condition does not involve a primary infection but rather a primary selective hemorrhagic mucosal necrosis with a moderate inflammatory reaction as a secondary phenomenon (5) In the hitherto published clinical studies the patients have typically been old or older individuals often with longstanding heart disease This condition does not seem to have been previously described in children or the newborn

Two cases of hemorrhagic mucosal necrosis observed in newborn infants are reviewed below One of these cases has previously been briefly mentioned in an article on this condition in the adult (5) Both of the newborn referred to here were only a few days old and in both cases intestinal perforation was seen in addition to hemorrhagic mucosal necrosis

## CASE HISTORIES

*Case I* (U 43515) The mother to this patient was a [illegible] para II gravida II The mother had had mild preeclampsia during her first pregnancy but delivered a normal child at term Delivery was uncomplicated

The mother again developed preeclampsia during her second pregnancy Because of decreasing estriol excretion in the urine Cesarean section was performed without complication five weeks before date of expected delivery The placenta was small and thin but contained no infarcts The child was a premature well-developed girl weighing 1400 g who did not present any signs of disease until two days after birth when increasing abdominal distension and peripheral cyanosis developed X ray of the abdomen showed a pneumoperitoneum Explorative laparotomy was performed on May 31 1966 The distal 4 cm of the ileum and the coecum appeared gangrenous and cloudy fluid was found in the peritoneum In addition a small perforation was seen in the gangrenous segment of ileum and therefore intestinal resection followed by an end-to-end anastomosis was carried out The child remained critical after operation and died during the first post-operative day

In the surgical specimen which consisted of a 45 mm long piece of the terminal ileum together with the coecum and the appendix a ragged-edged perforation measuring 12 4 mm was found The entire specimen was dark in color and edematous the coecum was markedly distended and contained a small amount of meconium

Microscopy revealed a hemorrhagic infarction limited to the mucosa which showed necrotic changes extreme hyperemia in the propria with interstitial bleeding and considerable edema There was only slight inflammation in association with the perforation I the submucosa, where the vessels were congested and dilatated moderate bleeding and edema but no inflammatory changes were found The muscularis was thin and extended but without necrosis.

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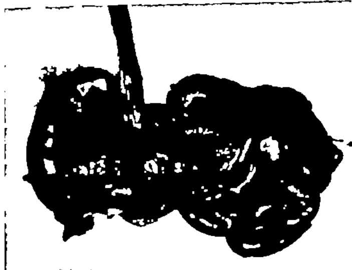


*Fig 2* Case 1 Marked dark discoloration of the entire gastrointestinal tract

pheral circulation became increasingly insufficient and the infant died during the second post operative day

On examination of the surgical specimen a 20 mm section of the terminal ileum the coecum and appendix and the ascending colon an irregular shaggy hemorrhagic perforation-opening was found in the coecum Fibrin deposition was seen around the perforation and the entire segment was dark red in color and appeared gangrenous There was no evidence of

*Fig 3* Case Microscopic picture of the intestine (surgical specimen) Sloughing of the mucosa is seen together with hyperemia and bleeding in the propria and marked hyperemia and edema in submucosa.



*Fig 1* Case 1 Microscopic picture of the intestine (surgical specimen) Hemorrhagic necrosis of the mucosa is seen together with moderate hyperemia and edema in the submucosa

Normal ganglion cells were present in the intestine (Fig 1)

On autopsy no peritonitis was found Pathological changes were seen throughout the gastrointestinal tract from the distal end of the esophagus to the anus consisting of a marked dark red discoloration of the mucosa Scattered small ulcerations and plump edematous mucosal folds were also present The intestinal contents were abundant and bloody but without meconium No stenoses or evidence of volvulus was seen The mesentery was normal in length and there were no occlusions of the arteries or veins The intestinal anastomosis appeared normal (Fig 2)

Microscopic examination of the gastrointestinal tract revealed changes of varying degree and of the same type as seen in the surgical specimen but with maximal intensity in the region of the anastomosis There were normal ganglion cells throughout the intestine On examination of the other organs scattered atelectases were found in the lungs The heart was normal in size and without malformations There was no evidence of mucoviscidosis or erythroblastosis

*Case 2* (J 50412) The mother to the patient was a 25 year old para I gravida I who had been hospitalized two weeks before term because of preeclampsia She developed labor pains a few hours after admission and delivered a normal male infant weighing 2800 g Birth was by breech presentation and Pitupartin® had been given during labor because of secondary delay The placenta was normal in size and without infarction

The infant presented no signs of illness before the second day of life when vomiting and gradual abdominal distension developed X ray of the abdomen showed a pneumoperitoneum On laparotomy (October 10 1966) the coecum was dark in color and contained a large perforation and therefore intestinal resection was performed The post operative course was uncomplicated during the first few days but on the sixth post operative day increasing distension of the abdomen again developed accompanied by peripheral cyanosis Laparotomy was again carried out on suspicion of anastomosal stenosis At operation a severe diffuse peritonitis was found together with a necrotic area measuring 1 × 1 cm at the site of the anastomosis Because of the severe peritonitis re anastomosis was not done but rather a diverting ileostomy and transversostomy After operation the peri

in the phase of stagnant anoxia just as in the dog experiments In this case the intestinal perforation would be idiopathic (18) It does not however appear likely that the hemorrhagic intestinal necrosis arose in case 2 during the phase of irreversible shock as the infant lived for one week after the first operation at which time the diagnosis hemorrhagic mucosal necrosis had been confirmed histologically One must therefore conclude from the information available that it is not possible to give an adequate explanation for the development of either the hemorrhagic mucosal necrosis or the intestinal perforation in the two cases presented in the present work

Intestinal perforation in the newborn is a rare condition with a high mortality (3 9 18) Most cases are caused by mechanical obstruction in the intestinal tract usually because of atresia severe stenosis or meconium plugs which are particularly common in mucoviscidosis (18 19) Other common causes are malrotation volvulus and torsion of the mesentery A few cases of perforation have been described in association with Hirschsprung's disease (11)

Intestinal perforation without mechanical occlusion is seen with congenital defects of the tunica muscularis (1) perforation of a diverticulum and rupture in areas of ectopic gastric mucosa with a peptic ulcer (18) There are only a few reports of intestinal perforation on a vascular basis and here mesenteric occlusion mycotic emboli with infarction and congenital arteriovenous anastomoses weakening the intestinal wall have been named as possible etiologic factors (16 17) Enteritis developing after the ingestion of infected amniotic fluid and necrotizing enterocolitis are given as causes of perforation resulting from a primary inflammatory condition (6 7 8 20) In many cases of intestinal perforation no cause can be found and these cases are therefore referred to as idiopathic or spontaneous (18)

As mentioned above hemorrhagic mucosal necrosis has not previously been reported in children However the possibility exists that some of the cases of idiopathic intestinal perforation described in the literature could actually have been cases of hemorrhagic mucosal necrosis or could have developed as a result of this condition especially as the reported cases have contained only a sparse description of the pathologic findings

Hemorrhagic mucosal necrosis seems to be a well defined histological entity The most predominant findings are marked hyperemia and edema in the mucosa and submucosa interstitial hemorrhage in the propria necrotic changes in the mucosa with sloughing (often in several areas) and the oozing of blood into the bowel lumen An inflammatory reaction is either absent or minimal The musculature presents no evidence of infarction With pronounced submucosal edema the musculature is stretched and the possibility of rupture is present Macroscopic as well as microscopic changes are found in the greater part of the intestinal tract but in varying degree The intestinal wall is normally structured and contains the normal number of ganglion cells Likewise the arteries and veins present no other changes than enormous congestion which results in diapedesis bleeding and the transudation of plasma into the surrounding tissues

Finally it should be mentioned that even though hemorrhagic mucosal necrosis presents a characteristic histological picture the etiology and pathogenesis of this condition in adults as well as in children have not been elucidated

## SUMMARY

Two cases of hemorrhagic mucosal necrosis in the gastrointestinal tract without vascular occlusion are reported This combination does not seem to have been previously described Both cases were observed in newborn infants and in both cases the condition was combined with intestinal perforation Both infants died Possible etiologic factors are discussed and the histological criteria reviewed

obstruction and no meconium was seen in the intestinal segment

On microscopic examination of the surgical specimen necrotic and mildly inflamed tissue with edema was found in association with the perforation and there was considerable bleeding in the mucosa and submucosa Hemorrhagic necrosis of the mucosa with hyperemia interstitial bleeding and edema was seen in the entire specimen In the submucosa there was likewise edema and appreciable vascular dilatation but only a mild inflammatory reaction The musculature was intact except at the site of perforation and the intestine contained ganglion cells throughout (Fig 3)

Autopsy revealed a marked purulent peritonitis with strongly adherent loops of bowel and a localized abscess under the right diaphragm Normal postoperative findings were seen in relation to the two intestinal stomata and there was no evidence of obstruction or congenital malformation The entire intestinal tract was characterized by the presence of spotty areas of poorly defined hemorrhage in the mucous membrane of the small intestine and most of the colon

On microscopic examination of the intestine changes of the same type as in the surgical specimen but less pronounced were noted The intestine contained no aganglionic areas Examination of the rest of the body revealed normally developed lungs evidencing acute stasis and mild edema together with scattered small atelectatic areas The heart was normal in size and without malformation There was no evidence of mucoviscidosis or erythroblastosis

## DISCUSSION

Hemorrhagic necrosis in the gastrointestinal tract is a typical finding in dog dying in irreversible shock regardless of whether it is hypovolemic endotoxic adrenalin or coronary shock According to Lillehei (10) this condition is seen in the phase of irreversible shock in the dog because of stasis in the splanchnic area which arises as a consequence of persisting post capillary vasoconstriction and termination of a preceding pre capillary vasoconstriction

Th etiology and pathogenesis of hemorrhagic mucosal necrosis in animals thus appears to be fairly well elucidated This is however not the case in humans Patients with this condition eem to fall into three groups (5 13 14) The majority of patients are older individuals with chronic heart disease who develop an abdominal illness often suggesting mesenteric thrombosis In a smaller number abdominal symptoms appear in connection with an infection Finally there are a few patients with primary shock often coronary where intestinal symptoms are usually either mild or totally lacking Patients in all three groups die as a rule in irreversible shock and only a few cases of recovery have been reported (5 12) Most investigators believe that this condition results from ischemic anoxia in the mesenteric area on the basis of a compensatory vasospasm (2 4 12) It has also been suggested that hemorrhagic mucosal necrosis is a consequence of a stagnant anoxia which arises during the phase of irreversible shock because of persisting post-capillary vasoconstriction in the face of a terminated pre-capillary vasoconstriction as in the dog If this is the case then intestinal symptoms are caused by the preceeding compensatory ischemic anoxia in the splanchnic area (5) The importance of digitalis as an etiologic factor because of the ability of this drug to increase vasoconstriction in the splanchnic area has been emphasized (15)

The two cases reported here both involved newborn infants This condition does not seem to have been previously described in patients in this age group Intestinal perforation was seen in both cases and in both cases changes typical of hemorrhagic mucosal necrosis were found in both the surgical and the autopsy specimens Whether the hemorrhagic mucosal necrosis or the perforation was primary cannot be determined If the hemorrhagic mucosal necrosis was primary then perforation could have been caused by the fact that the lamina muscularis is thin in the newborn and therefore could not withstand the intraluminal pressure in the bowel when pronounced changes were present in the mucosa and submucosa By accepting such a hypothesis one is left without a reasonable explanation for the development of the hemorrhagic mucosal necrosis If intestinal perforation was primary then the hemorrhagic mucosal necrosis could have been a secondary phenomenon appearing during shock

in the phase of stagnant anoxia just as in the ... experiments. In this case the intestinal perforation would be idiopathic (18). It does not however appear likely that the hemorrhagic intestinal necrosis arose in case 2 during the phase of irreversible shock as the infant lived for one week after the first operation at which time the diagnosis hemorrhagic mucosal necrosis had been confirmed histologically. One must therefore conclude from the information available that it is not possible to give an adequate explanation for the development of either the hemorrhagic mucosal necrosis or the intestinal perforation in the two cases presented in the present work.

Intestinal perforation in the newborn is a rare condition with a high mortality (3, 9, 18). Most cases are caused by mechanical obstruction in the intestinal tract usually because of atresia, severe stenosis or meconium plugs which are particularly common in mucoviscidosis (18, 19). Other common causes are malrotation, volvulus and torsion of the mesentery. A few cases of perforation have been described in association with Hirschsprung's disease (11).

Intestinal perforation without mechanical occlusion is seen with congenital defects of the tunica muscularis (1), perforation of a diverticulum and rupture in areas of ectopic gastric mucosa with a peptic ulcer (18). There are only a few reports of intestinal perforation on a vascular basis and here mesenteric occlusion, mycotic emboli with infarction and congenital arteriovenous anastomoses weakening the intestinal wall have been named as possible etiologic factors (16, 17). Enteritis developing after the ingestion of infected amniotic fluid and recurring enterocolitis are given as causes of perforation resulting from a primary inflammatory condition (6, 7, 8, 20). In many cases of intestinal perforation no cause can be found and these cases are therefore referred to as idiopathic or spontaneous (18).

As mentioned above hemorrhagic mucosal necrosis has not previously been reported in children. However the possibility exists that some of the cases of idiopathic intestinal perforation described in the literature could actually have been cases of hemorrhagic mucosal necrosis or could have developed as a result of this condition, especially as the reported cases have contained only a sparse description of the pathologic findings.

Hemorrhagic mucosal necrosis seems to be a well defined histological entity. The most predominant findings are marked hyperemia and edema in the mucosa and submucosa, interstitial hemorrhage in the propria, necrotic changes in the mucosa with sloughing (often in several areas) and the oozing of blood into the bowel lumen. An inflammatory reaction is either absent or minimal. The musculature presents no evidence of infarction. With pronounced submucosal edema the musculature is stretched and the possibility of rupture is present. Macroscopic as well as microscopic changes are found in the greater part of the intestinal tract but in varying degree. The intestinal wall is normally structured and contains the normal number of ganglion cells. Likewise the arteries and veins present no other changes than enormous congestion which results in diapedesis bleeding and the transudation of plasma into the surrounding tissues.

Finally it should be mentioned that even though hemorrhagic mucosal necrosis presents a characteristic histological picture, the etiology and pathogenesis of this condition in adults as well as in children have not been elucidated.

## SUMMARY

Two cases of hemorrhagic mucosal necrosis in the gastrointestinal tract without vascular occlusion are reported. This combination does not seem to have been previously described. Both cases were observed in newborn infants and in both cases the condition was combined with intestinal perforation. Both infants died. Possible etiologic factors are discussed and the histological criteria reviewed.

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CASE REPORT

# ACUTE HEMIPLEGIA IN GLYCOGEN STORAGE DISEASE TYPE I

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Glycogen storage disease (GSD) type 1 is an inborn error of carbohydrate metabolism resulting from the diminished activity of the enzyme glucose 6-phosphatase in the liver (1) kidney (4) and the small intestine (8) Although the disease is manifested by profound hypoglycemia with or without seizures residual neurologic impairment is an infrequent occurrence. This report describes the occurrance of acute hemiplegia followed by episodes of transient amaurosis in a 3 year-old child with GSD Type 1

## CASE REPORT

V W was born 1 7 65 to a 7 year-old white female gravida III para III abortions 0 at term A male sibling died at 2 years of a with GSD Type 1 confirmed by demonstrating diminished activity of glucose-6-phosphatase in an ante mortem liver biopsy

At 4 months of age the patient had a generalized seizure unaccompanied by fever Physical examination revealed a small white female with cherubic facies. The l er was palpable at the level of the umbilicus No enlargement of the spleen was noted Neurologic examination was normal

Random 3 and 4 hour post prandial blood glucose concentrations varied between 5 and 41 mg 100 ml Subcutaneous injection of glucagon (0 1 ml kg) failed to produce a rise in the blood glucose concentration but did produce a rise in the serum lactic acid level

The clinical picture of hepatomegaly hypoglycemia with seizures lactic acidosis, and flat glucagon tolerance test with concomitant elevation of the serum lactic acid level suggested a diagnosis of GSD Type 1

Between 5 and 15 months of age the patient was hospitalized 10 times seven times for seizures and 3 times for bilateral otitis media accompanied by fever and anorexia

The patient subsequently experienced no seizures until 21 months of age 31 10 66 when in the early morning she vomited became limp and remained comatose despite rectal dextrose The patient was unresponsive with perioral cyanosis shallow respirations and the right sided twitching of the face and extremities Following intravenous dextrose (40 ml of a 50% solution) the right sided twitching ceased respiratory excursions and the level of consciousness improved

Five hours later while receiving intravenous dextrose (10 solution) the patient had a clonic seizure involving the right arm and leg, which was controlled with 30 ml of 50% glucose intravenously and 2 ml of rectal paraldehyde The blood glucose concentration 1 hour prior to the seizure was 121 mg 100 ml The cerebrospinal fluid (CSF) contained 5 WBC/ mm a glucose content of 141 mg/100 ml and a protein content of 15 mg/100 ml No subsequent seizures occurred and the patient was discharged 10 days after admission on 9 11 66

Six days later (15 11 66) the patient was readmitted because of irritability anorexia lethargy and refusal to walk Physical examination revealed moderate right facial weakness and a right homonymous hemianopsia. The right arm was held in a semi flexed position the right leg was externally rotated and the gait was unsteady Muscle tone and deep tendon reflexes were equal bilaterally there were bilateral plantar toe signs In conjunction with the EEG (see below) a diagnosis of a left hemispheral cerebrovascular accident was made

During the next 1 months gradual improvement in the motor function of the right arm and leg occurred, and no seizures or symptoms of hypoglycemia were apparent with every 4 hour feedings.

On 28 11 67 the patient had an episode of amaurosis lasting / hour and followed by lethargy

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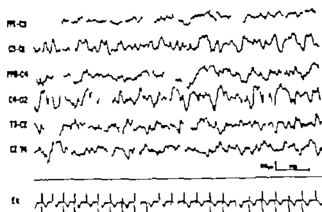


*Fig 3* 25 3 68 (17 months after the prolonged seizure) This is a resting waking record which shows excessive high voltage biocceipital delta slowing A late and persisting change in the patient's record

Patients with GSD Type 1 have a bleeding diathesis secondary to defective platelet adhesiveness (3) The hemiplegia could have been due to a focal cerebral hemorrhage resulting in homonymous hemianopsia with a persisting irritative focus responsible for subsequent episodes of blindness Hypoglycemia is associated with elevations in the plasma free fatty acid levels in patients with GSD Type 1 (2) and elevations in the plasma free fatty acid levels have been shown to initiate thrombotic episodes in various clinical conditions (6) Although thrombosis has not been reported in GSD Type 1 conceivably a prolonged seizure might have caused thrombus formation with resultant hemiplegia

Normal CSF without xanthrochromia or elevated protein content and the absence of red blood cells is more consistent with thrombosis than hemorrhage

Patients with idiopathic hypoglycemia and seizures have had hemiplegia (5) and residual seizure activity with normoglycemia (7) Presumably the normoglycemic seizures result from focal brain damage which develops during the hypoglycemic episodes

Because the transient blindness did not accompany seizure activity prior to the episodes of hemiplegia and because the seizures which occurred after the episode of hemiplegia developed when the patient was not hypoglycemic probably some permanent neural damage of the damaged hemisphere accompanied the seizure episode producting hemiplegia The response to mephobarbital would substantiate this interpretation

## SUMMARY

A 21 month old girl with GSD Type 1 developed acute hemiplegia with homonymous hemianopsia following a prolonged hypoglycemic seizure There was gradual improvement in motor function However the patient had subsequent episodes of transient blindness with and without seizure activity which were unrelated to hypoglycemia Serial EEG findings suggested that the hemiplegia resulted from a vascular accident and led to neural dysfunction causing subsequent transient blindness

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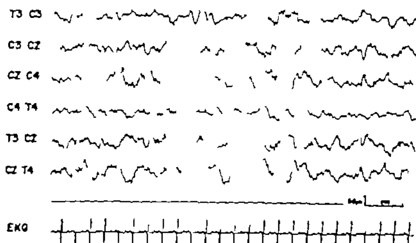



*Fig 1* 18 6 65 This is a normal sleep tracing showing diffuse symetrical slowing with synchronous and symetrical fourteen cycle per second sleep spindles (A baseline record taken prior to the acute hemiplegia)

There was no prodrome and the blindness occurred about 1 to 2 hours post prandially

Between 12 67 and 3 68 the patient experienced two episodes of vomiting lethargy and ataxia without blindness or seizure activity and one episode accompanied by blindness and aphasia which spontaneously remitted with rectal dextrose

On 24 3 68 while eating breakfast the patient complained of total blindness and experienced a clonic seizure involving the right leg and accompanied by coma Despite rectal glucose the seizure continued for 1 hour and only remitted after the intravenous administration of 10 ml of 50 dextrose solution The blood glucose value prior to intravenous dextrose therapy was 31 mg/100 ml Lethargy semi coma and blindness persisted for 6 to 12 hours and then resolved completely An EEG (see below) demonstrated bioccipital involvement with left predominance A brain scan following the intravenous injection of 500 microcuries of 197 Hg labelled Neohydrin was normal Mephobarbital 32 mg three times per day was started and in the next 10 months no subsequent episodes occurred

## ELECTROENCEPHALOGRAPHIC (EEG) FINDINGS

Serial EEG tracings are shown in Figs 1 3 The initial normal sleep (Fig 1) tracing was obtained prior to the initial episode of hemiplegia Fig 2 shows suppression of sleep spindles and excessive slow wave activity on the affected side after the hemiplegic episode Fig 3 shows excessive diffuse slowing posteriorly which was slightly more prominent in the left hemisphere in the tracing The decreasing abnormality with time was interpreted as consistent with a vascular accident

## DISCUSSION

The etiology of the patient's initial seizure activity prior to the onset of hemiplegia with resultant episodes of blindness was presumably hypoglycemia However the etiology and pathogenesis of the hemiplegia are speculative

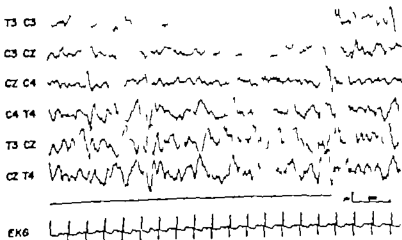



*Fig 2* 8 11 66 (8 days after the prolonged seizure) This demonstrates a diffuse slow wave activity during mid phases of sleep with asymetry of spindling The sleep spindles are obscured in the left hemisphere This finding was felt to be most consistent with a vascular lesion

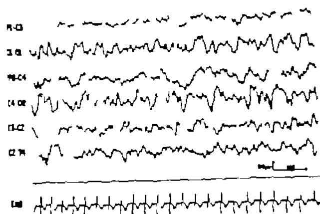


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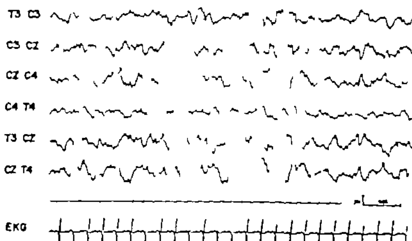



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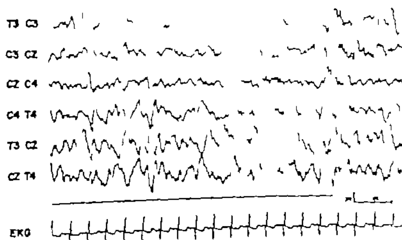



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*Acta Pædiat Scand 58 625–631 1969*

CASE REPORT

# GONADAL DYSGENESIS WITH LYMPHOCYTIC THYROIDITIS AND DELETION OF THE LONG ARM OF THE X CHROMOSOME

KERSTIN MELIN and GÖSTA SAMUELSON

From the Department of Paediatrics University Hospital Umeå Sweden

The combination of gonadal dysgenesis and thyroiditis has been reported during recent years *Williams et al* found an increased incidence of thyroid antibodies and clinical lymphocytic thyroiditis as well as primary myxoedema in 25 female patients with gonadal dysgenesis (27)

To our knowledge seven cases of lymphocytic thyroiditis associated with gonadal dysgenesis have so far been published (15 19 26 27) Two were children 9 and 10 years old respectively

Primary non goitrous hypothyroidism in gonadal dysgenesis was reported by de la Chapelle in a 14 year old girl and by Frey & Hoffman in a 16-year old girl (5 13) Some adult cases have also been described (27) Most of the cases with lymphocytic thyroiditis have had a chromatin positive X/iso-X karyotype (15 6 27) i e a duplication of the long arm of one X chromosome (46,XXqi) However Hutchison (19) reported one sex chromatin negative XO case (45,X)

We present here a girl with gonadal dysgenesis lymphocytic thyroiditis and a 45,X/46,XXq constitution [1]

## CASE REPORT

*H L* a girl born November 23 1951 was first admitted to the Department of Paediatrics University Hospital of Umeå in 1965 when she was 13 years old, because of short stature (Fig 1 a)

[1] Nomenclature of the Chicago Conference 1966 (6)

Her brother and both parents were healthy and showed no evidence of thyroid disorder Their respective body heights were within the normal limits The maternal grandmother was definitely of short stature and had since many years an enlargement of the thyroid The patient's birth weight was 3140 g Height 43 cm Pregnancy delivery and neonatal period were normal She had always been of short stature (Fig 2)

Physical examination at 14 years of age revealed that she was short necked had a low posterior hair line and cubitus valgus Body height was 133 cm Weight 45 8 kg The skin was dry and cold She had no secondary sex characteristics The thyroid was not palpable Auscultation of the heart revealed an innocent murmur ECG was normal Blood pressure 150/120–140/90 Femoral pulses were palpable Ophthalmological examination including an anomaloscope investigation showed colour amblyopia (green red quotient between 2 and 1 3) Otological examination and audiogram were normal and likewise a dental examination X ray examinations of the skeleton did not show any discrepancy between the chronologic and bone ages The epiphyseal lines in the knee joints were not closed X ray of the heart lung and skull was normal Intravenous pyelography revealed a horseshoe kidney

Gynaecological examination in November 1967 revealed ordinary external genitalia The breasts were underdeveloped and the pubic hair scanty The ovaries were considered definitely smaller than normal for the age at laparoscopy On the surface of the right ovary there was a roughness suggesting a previous ovulation

Routine haematological and urinary analyses were normal Serum cholesterol 505 *mg 100 ml*

PBI 0 5–0 9 μg/100 ml Triiodothyronine uptake in erythrocytes 9 4 11 0% Uptake of radioactive iodine by the thyroid gland was low (1% after 2 hours, 2 5% after 24 hours) and excretion in the urine was high A scintigram of the thyroid showed a minimal uptake over the gland and no change in uptake after stimulation with TSH Thyroid antibodies were detected in the serum (Table 1)

Platelets fatty acids and thrombosis *Circulation Res* 20 11 1967
7 Ingram T T S Stark G D & Blackburn I Ataxia and other neurological disorders as sequels of severe hypoglycemia in childhood *Brain* 90 851 1967
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To our knowledge seven cases of lymphocytic thyroiditis associated with gonadal dysgenesis have so far been published (15 19 26 27). Two were children 9 and 10 years old respectively.

*Primary non goitrous hypothyroidism in gonadal dysgenesis* was reported by de la Chapelle in a 14 year-old girl and by Frey & Hoffmann in a 16-year-old girl (5 13). Some adult cases have also been described (27). Most of the cases with lymphocytic thyroiditis have had a chromatin positive X/iso-X karyotype (15 26 27) i.e. a duplication of the long arm of the X chromosome (46 XXqi). However Hamilton (19) reported one sex chromatin negative X0 case (45,X).

We present here a girl with gonadal dysgenesis lymphocytic thyroiditis and a 45 X/46 XXq− constitution [1].

## CASE REPORT

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Her brother and both parents were healthy and showed no evidence of thyroid disorder. Their respective body heights were within the normal limits. The maternal grandmother was definitely of short stature and had since many years an enlargement of the thyroid. The patient's birth weight was 3140 g. Height 43 cm. Pregnancy delivery and neonatal period were normal. She had always been of short stature (Fig 2).

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Gynaecological examination in November 1967 revealed ordinary external genitalia. The breasts were underdeveloped and the pubic hair scanty. The ova*ries were considered definitely smaller than normal* for the age at laparoscopy. On the surface of the right ovary there was a roughness suggesting a previous ovulation.

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PBI 0.5–0.9 µg/100 ml. Triiodothyronine uptake in erythrocytes 9.4–11.0%. Uptake of radioactive iodine by the thyroid gland was low (1% after 2 hours, 2.5% after 24 hours) and excretion in the urine was high. A scintigram of the thyroid showed a minimal uptake over the gland and no change of uptake after stimulation with TSH. Thyroid antibodies were detected in the serum (Table 1).

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thyroiditis was obvious and treatment with daily doses of 0.15 m thyroxine (Levaxin®) was instituted in January 1965. The girl improved clinically on this therapy. All earlier signs of hypofunction of the thyroid disappeared and the thyroid functioning tests (PBI, BEI, total iodine and triiodothyronine uptake in the erythrocytes) were normalized.

The girl had menarche spontaneously in November 1964. The menstruations were small and irregular for the first three years but since January 1968 she has menstruated every month without oestrogen substitution. There has been a slight breast development but the pubic hair is still scanty (Fig. 1 b). She is still of short stature below −2 s.d. She has grown 7 cm during the last three years. X-ray of the skeleton has shown that the epiphyseal proximal tibial lines are partly closed and the epiphyseal line in the distal part of femur is to a greater part open.

The psychological development has been quite normal. Intelligence tests according to Terman-Merrill gave an IQ of 97–102. She has just terminated the nine year elementary school.



Fig. 2. Growth diagram of the patient.

*Family investigations*

Chromosome analyses of the parents revealed normal chromosome constitutions. There were no signs of mosaicism or structural chromosome aberrations in

Table 1. *Results of thyroid antibody studies*

| Subjects | Age | Thyroglobulin: Passive hemagglutination | | Cytoplasmic antigen: IFL | Cytoplasmic antigen: CFT[b] | Antinuclear factor: IFL |
|---|---|---|---|---|---|---|
| Patient H. L. | 17 | < 1/12.5 | neg | Pos | Pos 1/20 | Neg |
| Father E. L. | 43 | < 1/12.5 | neg | Neg | — | Neg |
| Mother I. L. | 40 | 1/3,200 | pos | Pos | Pos 1/10 | Neg |
| Brother S. L. | 15 | < 1/10 | neg | Neg | — | Neg |
| Maternal grandmother C. K. | 76 | < 1/100 | neg | Neg | | Neg |

[a] Immuno-fluorescence.
[b] Complement fixation titers.



Fig. 3. Thyroid gland showing lymphoid hyperplasia and increased fibrosis.

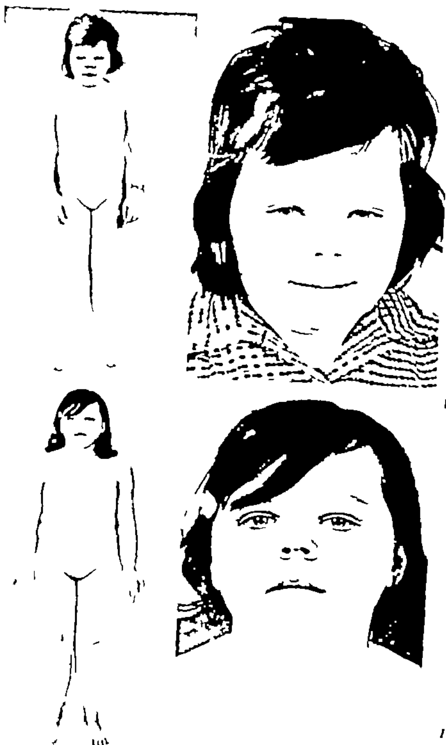
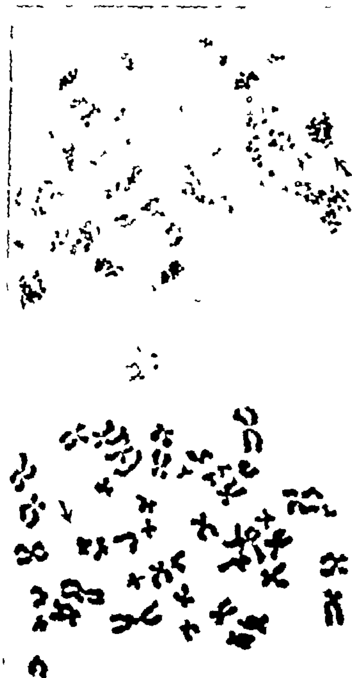



*Fig 1* The patient at age 13 (*a*) and 17 years (*b*)

Hormone analysis in the urine showed an increased excretion of total gonadotrophins more than 96 ME 24 hours Gonadotrophin analysis in September 1968 was normal The excretion of oestron oestradiol was 0 microgram/24 hours and of oestriol 14 4 microgram/24 hours These values are within the normal adult limits The excretion of 17 keto and 17 OH steroids were always within the normal variation Thin needle aspiration of the thyroid did not give sufficient material for an histopathological diagnosis A surgical biopsy was performed Macroscopically the thyroid appeared small and atrophic Microscopic examination showed lymphoid hyperplasia with a dominance of lymphocytes and also increased fibrous tissue (Fig 3)

Buccal mucosa smears showed a low frequency (3–7 ) of sex chromatin positive cells Chromosome analysis gave the following results out of 82 analysed cells from lymphocyte cultures eight had a 45 X and 74 a 46 XXq constitution (Fig 4) The deleted X chromosome was clearly late replicating at autoradiography (Figs 5–6) Analysis of the Xg blood group demonstrated that the girl was Xg (a+)

The diagnosis of gonadal dysgenesis and hypo



*Figs 5–6* Autoradiographs.

Similar cases have been reported by others (9 26) and the low titers may be explained by the progression and burning out of the thy[roid]itis with a decline of the thyroid antibodies [T]he findings of thyroid antibodies in the mother of our patient as well as the goitre in the maternal grandmother are not surprising An increased frequency of thyroid antibodies goiter and thyrotoxicosis may be found in parents siblings and other relatives of patients

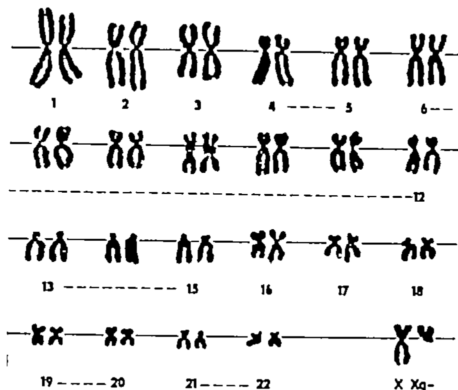



*Fig 4* The karyotype of the patient demonstrating a structural changed X chromosome with deletion of the long arm

the analysed blood cultures Both parents were Xg (a+)

Thyroid antibodies were detected in the serum of the mother but not in those of other relatives of the patient (Table 1)

The mother father brother and the maternal grandmother had normal colourvision The maternal grandfather deceased probably had a defect colour vision according to the family members

## DISCUSSION

The present girl is of short stature and has some of the stigmata typical of gonadal dysgenesis, such as cubitus valgus short neck low posterior hair line and horse shoe kidney

She had menarche spontaneously at 13 years of age After a period of irregular and sporadic menstruation she has now menstruated regularly for one year Similar cases of gonadal dysgenesis with spontaneous and regular menstruations have previously been described (5 11, 19 22) According to Ferguson-Smith menstruations were found in 8% of patients with a 45 X karyotype, in 20% of those with a 45,X/46 XX mosaics and in cases with other chromosome aberrations in varyingly low per cent Bahner *et al* (1) reported a Turner patient with a 45 X karyotype who gave birth to a normal child Our case was found to have small hypoplastic ovaries The observed rough surface on one ovary could indicate a previous ovulation

Primary non goitrous hypothyroidism and lymphocytic thyroiditis have been reported in patients with gonadal dysgenesis Our patient has hypothyroidism caused by a lymphocytic thyroiditis It is interesting to note that she has never had any visible or palpable goiter which is often but not consistently found in lymphocytic thyroiditis (23) In lymphocytic thyroiditis there appears to be variants from a well compensated goitre to an atrophic gland with hypothyroidism (3 29) and primary non goitrous hypothyroidism may be the end result of severe lymphocytic thyroiditis (8 24 29)

In lymphocytic thyroiditis antibodies against various components of thyroid tissue are commonly found (8 24) Our patient had such antibodies but only to cytoplasmic antigen

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*Key words* Gonadal dysgenesis thyroiditis deletion of the long arm of the X chromosome

with lymphocytic thyroiditis (9, 16 17) Familial occurrence of lymphocytic thyroiditis has also been reported (10)

The cytogenetical investigations demonstrated that the girl was sex chromatin positive in buccal mucosa smears The chromosome analysis showed a mosaicism with two cell lines one with 45 chromosomes and only one X and the other with 46 chromosomes with one normal X and one X chromosome with a deletion of the long arm This is an unusual chromosome aberration In a review of 307 case of gonadal dysgenesis Ferguson Smith (1965) reported only ten patients with structural abnormality of the long arm of one of the X chromosomes (11) Five of these ten patients had a deletion of the long arm without apparent mosaicism 46 XXq⁻ (2 7 11 14 20) One had a mosaic with a normal chromosome constitution but a deletion of the long arm 46 XX/46 XXq⁻ (14) Four had the same karyotype as our patient 45 X/46 XXq⁻ (4 7 11) and two of them had a typical Turner s syndrome including marked short stature In earlier published cases of Turner s syndrome with short stature either a 45 X constitution and/or a structural aberration of the X chromosome have been described but in all cases a deletion of the short arm is observed It has been assumed by Ferguson Smith (11) that monosomy of the short arm of the X chromosome is the determinating factor for short stature in Turner s syndrome Also Hamerton (18) suggests that controlling centra for growth are localized on the short arm of the heterochromatic X chromosome The short stature of our case can thus be attributed to the 45,X cell line

Other disorders with possible autoimmune etiology as ulcerative colitis and Addison s disease have also been described in association with gonadal dysgenesis (13 28) Among other not autoimmune disorders Forbes & Williams have reported a high incidence of diabetes mellitus in adult patients with gonadal dysgenesis (12)

The genes for deutan colour blindness and the blood group Xg have been assumed to be located on the short arm of the X chromosome (21 22) Contradictory conclusions however have been drawn by Polani *et al* (25) They investigated a family where the Xg locus appears to be placed on the long arm The present girl and both her parents were all Xg (a+) Therefore no conclusion could be drawn from where the abnormal chromosome had derived

Out of three cases with gonadal dysgenesis and primary nongoitrous hypothyroidism two were chromatin negative 45 X karyotypes while the third was chromatin positive but not chromosome analysed (5 13 27)

Most cases of gonadal dysgenesis with lymphocytic thyroiditis so far reported have been sex chromatin positive and have had a 46 XXqi karyotype Thus five out of seven hitherto published patients have had this chromosome constitution whereas one was chromatin positive with 45 X/46,XXr mosaic and one was chromatin negative with a 45 X karyotype (15 19 26 27) Our patient is another variant being sex chromatin positive with a 45 X/46 XXq⁻ mosaic

We are inclined to agree with Hamilton (19) and Buchanan (3) that lymphocytic thyroiditis and primary non goitrous hypothyroidism might be regarded as variants of the same autoimmune process which occurs with unusually high frequency in gonadal dysgenesis

## SUMMARY

A 17 year-old girl with several stigmata of gonadal dysgenesis and a 45 X/46 XXq⁻ mosaicism is presented She had small hypoplastic ovaries and regular menstruations and a lymphocytic thyroiditis The relationship between gonadal dysgenesis and lymphocytic thyroiditis is discussed

## ACKNOWLEDGEMENTS

The authors are much indepted to Drs M Fraccaro and L Tiepolo Pavia Italy for their help with the autoradiographic analysis Drs R Race and Ruth Sanger London England for the analysis of the Xg blood group and to Dr J Lindsten Stockholm Sweden for the analysis of the chromosome in cells from the peripheral blood

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*Acta Pædiat Scand 58 632–637 1969*

CASE REPORT

# COARCTATION OF THE AORTA WITH MULTIPLE ARTERY ANOMALIES IN IDIOPATHIC HYPERCALCEMIA OF INFANCY

J BLIDDAL B DUPONT J C MELCHIOR and O E OTTESEN[1]

*From the Cardiovascular Laboratory Department of Medicine B Department of Radiology and Department of Paediatrics Rigshospitalet University Hospital Copenhagen Denmark*

The occurrence of idiopathic hypercalcemia in infancy (IHI) with supravalvular aortic and pulmonary artery stenosis hypoplasia of the aorta and multiple stenoses of the peripheral arteries is now a well known entity (7 15 20) This paper describes in the same patient the above mentioned anomalies in association with coarctation of the aorta A combination which to our knowledge has not previously been reported

## CASE REPORT

Patient J N H a 4 / years old boy was first admitted to the Department of Pediatrics University of Copenhagen at the age of eleven months because of failure to thrive vomiting obstipation and for evaluation of a systolic murmur of the heart

The family history revealed that an uncle had slight mental retardation his appearance was normal The patient was the third of three siblings the other two were healthy The pregnancy and the delivery were uncomplicated and the mother did not take extra vitamin D during pregnancy The birth weight was 3250 g and the length 53 cm The child was breast fed for only three days thereafter he was bottle fed on diluted cows milk From four months of age he was given undiluted cows milk and started vomiting and did not gain weight He was definitely retarded and uninterested in his surroundings At nine months of age he was not able to sit unsupported

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On the admission at eleven months of age the weight was 7600 g the length 74 cm The face was elfin like with high prominent fore head broad depressed nasal ridge and coarse pouting lips There was concomitant strabismus on the left eye Auscultation of the heart revealed a systolic Grade II murmur along the left sternal border with radiation to the back and the axilla Hypotonia with normal tendon reflexes was demonstrated The ECG showed right axis deviation The chest roentgenogram revealed the heart to be borderline widened with elevation of the apex and a suggestion of right ventricular enlargement Right heart catheterization demonstrated a pressure in the right pulmonary artery of 15/7 mm Hg with an increase in the main pulmonary artery to 72/6 mm Hg and with no gradient across the pulmonary valves Angiocardiography was performed with injection of contrast medium in the right ventricular outflow tract The examination revealed a type II B stenosis of the pulmonary truncus and arteries (6) involving the main pulmonary trunc in association with stenosis of the origin of the right and left pulmonary branches and hypertrophy of the right ventricle

From the age of 2 / to 4 / year the patient was admitted several times for further investigations Hypercalcemia (13 6 to 10 6 mg per 100 ml serum) was demonstrated at 2 / years of age and was successfully suppressed for 1 / year with cortisone After this period the serum calcium was normal Psychological examinations revealed that the patient was mentally retarded (debile) Pyuria was demonstrated but intravenous urography revealed no signs of nephrocalcinosis However the right kidney was rotated and situated at the level of the 3rd and the 4th lumbal vertebrae The excretion time was normal but the concentration of the contrast medium was poor No other abnormalities were noted at this ex



*Fig 1* Photograph of the patient 4 1/12 years old showing characteristic facies

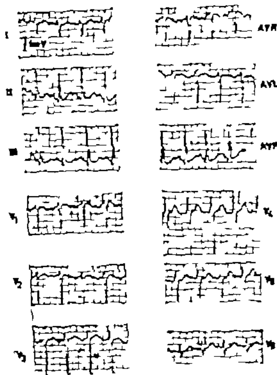



*Fig 2* ECG of the patient when 4 1/12 years old

mination Because of the recidivating pyuria a micturition cystourethrography was performed This examination demonstrated bladderneck obstruction diverticula formation and trabeculation of the bladder

On the last admission to the pediatric department in August 1968 the child demonstrated slight mental retardation There were no gastro-intestinal symptoms no dyspnoea on exertion no oedema and no cyanosis nor did he complain of chest pain or headache The blood pressure in both arms was 230/130 mm Hg The facial appearance was still elfin like (Fig 1) Examination of the eyes demonstrated concomitant strabismus of the left eye Ophthalmoscopic examination revealed Grade I changes Auscultation of the heart was unchanged A high frequency systolic murmur was audible over the midline of the abdomen A high amplitude pulse was present in the neck and both upper extremities the femoral pulses were weak bilaterally

The plain chest radiograph demonstrated enlargement of the heart with an accentuated vascular markings in the lung fields The aortic knob was absent and rib notching was demonstrated bilaterally The electrocardiogram showed right axis deviation (Fig 2)

The neurological investigation demonstrated normal tendon reflexes and muscular tonus and no pare

## INVESTIGATIONS

The hemoglobin was 12 9 g per 100 ml the serum-creatinine 0 8 mg per 100 ml the serum calcium 10 3 mg per 100 ml serum phosphorus 4 9 mg per 100 ml the urine revealed no protein or leucocytes Quantitative culture of

Table 1 *Cardiac catheterization data*

*The cardiac catheterization was performed during fluothane anaesthesia. The blood pressure in the arms before and after the anaesthesia was 230/130 mm Hg, but during the anaesthesia there was a marked blood pressure fall*

| | Pressure in mm Hg | | |
|---|---|---|---|
| Catheter position | Systolic | Diastolic | Mean |
| Right ventricle | 37 | 4 | |
| Main pulm artery | 37 | 12 | |
| Left pulm artery | 17 | 11 | 15 |
| Right pulm artery | 16 | 11 | 14 |
| Pulmonary "capillary" wedge | | | 11 |
| Left ventricle | 115 | 15 | |
| Aortic arch | 110 | 53 | |
| Descending aorta distal to coarctation | 54 | 39 | |

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*Fig. 6.* Abdominal aortogram (antero-posterior projection) with stenosis of the renal arteries and poststenotic dilatation.



*Fig. 7.* Abdominal aortogram (lateral projection) with stenosis of the coeliac artery and the superior mesenteric artery.

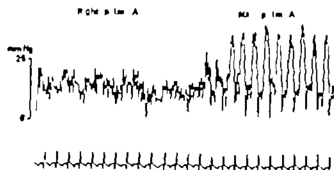


*Fig 3* Pressure recordings demonstrating the supravalvular pulmonary artery stenosis

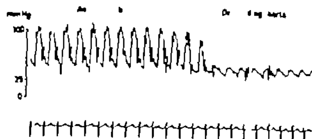


*Fig 4* Pressure recordings demonstrating the pressure gradient across the aortic coarctation

the urine revealed more than 10 proteus vulgaris per ml

A right heart catheterization was performed The findings are shown in Table I The stenosis of the right and left pulmonary artery was confirmed with a mean systolic gradient of 20 mm Hg (Fig 3) No shunt was demonstrated on oxygen saturations The pressure in the right ventricle was elevated to 37/4 mm Hg

A retrograde left ventricular catheterization from the right femoral artery was performed during fluothane anaesthesia The blood pressure in the arms before and after the anaesthesia was 230/130 mm Hg but during the anaesthesia there was a marked fall in the blood pressure As demonstrated in Table 1 a coarctation of the aorta was revealed just distal to the origin of the left subclavian artery with a pressure gradient of 55 mm Hg systolic and 24 mm Hg diastolic (Fig 4) No gradients across the aortic valve or in the ascending aorta were demonstrated During the catheterization thoracic and abdominal aortography was performed

The thoracic aortogram demonstrated left ventricle hypertrophy The aortic valves, three in number were normal A moderate degree of supravalvular aortic stenosis, hypoplasia of the aortic arch and a coarctation of the descending aorta located distal to the origin of the left subclavian artery were noted The coarctation was 1 5 cm in length Numerous tortuous, dilated collateral vessels including the intercostal arteries and the internal mammary arteries were seen as well (Fig 5)



*Fig 5* Thoracic aortogram (left posterior projection) with supravalvular aortic stenosis and coarctation of the descending thoracic aorta

cribed This patient demonstrated a combination of supravalvular pulmonary artery stenosis supravalvular aortic stenosis hypoplasia of the aortic arch coarctation of the aorta and multiple stenoses of the branches of the abdominal aorta The pathogenesis of arterial hypertension in this syndrome is discussed

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*Key words* idiopathic hypercalcemia of infancy mental retardation arterial hypertension coarctation of the aorta coeliac artery stenosis, mesenteric artery stenosis renal artery stenosis supravalvular aortic stenosis supravalvular pulmonary artery stenosis

The abdominal aortogram demonstrated multiple stenoses of the aortic branches i e the coeliac axis superior mesenteric artery and renal arteries Both kidneys were supplied by two arteries The right aberrant renal artery originated just proximal to the aortic bifurcation All abdominal arterial branches demonstrated some degree of post stenotic dilatation (Figs 6 and 7)

## DISCUSSION

Idiopathic hypercalcemia of infancy (IHI) with elfin face mental retardation failure to thrive and multiple congenital abnormalities was first described by Fanconi & Schlesinger in 1952 (5) Patients with the same characteristic elfin face mental retardation failure to thrive in infancy and congenital anomalies in the arteries but without demonstrated hypercalcemia, are now regarded as the idiopathic hypercalcemia of infancy syndrome (IHIS) The arterial abnormalities reported thus far include supravalvular aortic stenosis (19) supravalvular aortic and supravalvular pulmonary artery stenoses (2 7) and supravalvular aortic stenosis, hypoplasia of the aorta and multiple arterial stenoses of the aortic branches (14 20) One patient with verified IHI and interruption of the aortic arch is reported (8) In 1966 Blancquaert *et al* published 10 cases with IHIS (3) In two of their patients a coarctation of the aorta was demonstrated One patient had pulmonary hypertension but no other cardiovascular abnormalities were demonstrated The other patient had a supravalvular pulmonary stenosis Najafi *et al* (12) reported one patient with IHIS who had supravalvular aortic stenosis and coarctation of the aorta Barold *et al* (1) has recently presented a patient with the elfin face mental retardation and coarctation of the aorta, but without supravalvular aortic stenosis or pulmonary artery stenosis This patient had a blood pressure of 120/70 mm Hg There was a mean systolic gradient of 28 mm Hg across the coarctation In none of the four patients with the syndrome and coarctation of the aorta investigation of the abdominal arteries was made and in none of them were hypercalcemia periods demonstrated Sutcliffe (18) reported two cases of IHI with coarctation of the aorta In one of these the blood pressure was 180/100 mm Hg and in this patient, an abdominal aortogram revealed stenosis of the right renal artery In these cases the gradients across the coarctation were not measured

Coarctation of the aorta is commonly associated with other cardiovascular anomalies such as a persistent ductus bicuspid aortic valves valvular aortic stenosis and hypoplasia of the aortic arch The combination of supravalvular aortic stenosis and coarctation of the aorta is reported in eight patients without IHIS (9 10 11 13 16 21) To our knowledge there is no previous report of the association of multiple stenoses of the abdominal arterial branches and coarctation of the thoracic aorta

A number of the patients with the IHIS have arterial hypertension The pathogenesis of the hypertension has been attributed to chronic pyelonephritis with nephrocalcinosis although the nephrocalcinosis was only demonstrated radiologically in a few cases (4 17) The hypertension might in some patients be secondary to renal artery stenosis and in some to coarctation of the aorta

The patient described in this report has well documented IHI He has the typical elfin face mental retardation and the combination of supravalvular pulmonary artery stenosis (Type II B) slight supravalvular aortic stenosis coarctation of the aorta with hypoplasia of the aortic arch and stenosis of the coeliac mesenteric and renal arteries The patient has arterial hypertension and coarctation of the aorta with well developed collateral circulation

He demonstrates the importance of investigating not only the thoracic aorta but the abdominal aorta as well in patients suspected for IHIS

## SUMMARY

A case of idiopathic hypercalcemia of infancy with multiple anomalies of the arteries is de

## Meeting Oct 26 1968

; Sterky & C Thoren *Heart volume in over eight children*

Heart volume related to kg bodyweight has in normal boys an average value of 11 5 ml kg which agrees closely with the 11 6 ml which Kjellberg & Sjostrand found for adults

In 70 schoolboys aged about 12 years with excess weight varying between + 2 and + 4 standard deviations the relation of heart volume to work capacity and body size was studied The maximum oxygen intake capacity showed a good correlation to heart volume as did work capacity expressed as $W_{1\,4}$ The best correlation however was that with the fat free body weight which is an expression of the muscle mass

Schwalb & Eberl (1967) showed that adult men aged 30–50 who were only 10–20 over weight had a larger heart volume in relation to body length and that heart volume increased with weight increase This apparently also applies to boys before puberty With slight to moderate overweight there is to begin with an adaptation of the functional dimensions to the increased body weight so long as the person is able to move about sufficiently Increasing inactivity however leads to an increase in over weight, while work capacity and heart volume diminish This leads to an increase in the physical effort required which results in a further increa e in inactivity The vicious circle must therefore be broken in time

## Meeting Oct 12 1968

L Kohler H E Holst, Kerstin Holst Eva Mari Kohler G Stigmar & B Lindquist *Health control of 4 year old children Preliminary results of a pilot study*

*Introduction* (B Lindquist) The aim of this study was to determine prevalence of different types of handicaps and to evaluate various parameters for screening and thereby lay the basis to the planned general health control of 4 year-old children in Sweden

Data on past and family history was obtained from que tionnaires filled in by the parents As a rule these were properly filled in

The investigation comprised the following (1) Physical examination with neurological evaluation by a pediatrician (2) vision and hearing tests performed by nurses (3) dental examination by a dentist (4) mental health and social adaptation by a psychologist The first two investigations were done on the first visit and the dental and psychological evaluations on the second and third visit, respectively Items 1 2 and 4 were performed at the Child Health Center and item 3 at the Public Dental Clinic

All 4 year old children in the town of Lund in 1967 were included Of these 672 94 6 attended 20 could not be contacted and 16 families refu ed to allow their children to be examined Judging from other available information these children do not have a proportionately greater number of handicapping conditions

*Physical examination* (L Kohler) This examination took approximately 20 minute and included a physical examination with neurological creening and some laboratory tests (Hgb screening for bacteriuria) Of the 636 children who participated in the health control 624 (98 ) could be completely examined In 32 of these children one or more previously unknown physical defects were detected most of them mild—e g flat foot narrow foreskin Ten per cent were referred to different specialists for further examination

Twenty four children (3 8 ) were referred to a *pediatrician* 15 because of neurological disturbances Of these five were found to have cerebral dysfunction one a chronic peripheral neuropathy one a benign central core di ease

# PROCEEDINGS OF PAEDIATRIC SOCIETIES

## SWEDISH PAEDIATRIC SOCIETY

### Meeting May 22, 1968

L Hambraeus & L Wranne *Aspects of diagnosis and treatment of homocystinuria*

Two cases of homocystinuria were studied The patients a boy aged 6 years and a girl aged 4 ½ years were siblings The boy showed the typical appearance associated with homocystinuria but his sister's appearance was only slightly changed

Two different types of treatment were tested one being based on reduction of intake of the sulphur amino acid methionine the other being based on administration of pyridoxine in large doses as this might stimulate the defect enzyme cystathionine synthetase

It was shown that a lentil diet low in sulphur containing amino acids elicited a prompt decrease in serum and urinary methionine and homocystine in both cases the latter substance being no longer detectable on the fourth post treatment day

The administration of pyridoxine 300 mg daily for a period of 6 months resulted in a decrease in the serum concentration and to a lesser degree also in the urinary output of methionine in both cases Serum homocystine decreased slightly in the boy but remained unchanged in his sister The urinary level of homocystine showed no change during therapy

T Berg & Gunnar O Johansson *The development of immunoglobulin levels during the first year of life a longitudinal study*

In the present investigation 33 normal infants had *serial* determination of immunoglobulins during the first year of life The samples were obtained on the first day at the age of three week and subsequently at the same age as in the infants described in a previous report (*Acta Paediat Scand* 56 572 1967) Twelve infants had no infections and will not be discussed in this report The remaining 21 infants had a higher than average incidence of infections Five of them have had recurrent infection of the upper respiratory tract otitis bronchitis etc None of the infants in this series has shown any other symptoms or has had any serious illness

*Results IgG* The average IgG levels in the 21 infants did not differ remarkably from those found in the previous study The five infants with frequent infections tended to have higher IgG concentrations during the first few weeks of life Infants with the lowest IgG concentrations showed an increase over the same period This finding may indicate a later start of IgG synthesis in infants having a high serum concentration of maternal IgG at birth

*IgA* IgA levels in the infants of the present serial study showed constantly higher mean concentrations from about 6 months of age than in children of the earlier series The five infants with recurrent infections had higher IgA levels during the entire period of observation and had more than twice as high a concentration of IgA at the age of one year as infants in the previous series

*IgM* The mean concentrations of IgM were consistently higher in infants with frequent infections and high concentrations of IgM were observed at a very early age At the age of one year infants with recurrent infections had on the average more than twice the IgM concentration of infants with few infections

## Meeting Oct 26 1968

G Sterky & C Thoren *Heart volume in over weight children*

Heart volume related to kg bodyweight has in normal boys an average value of 11 5 ml/kg which agrees closely with the 11 6 ml which Kjellberg & Sjostrand found for adults

In 20 schoolboys aged about 12 years with excess weight varying between +2 and +4 standard deviations the relation of heart volume to work capacity and body size was studied The maximum oxygen intake capacity showed a good correlation to heart volume as did work capacity expressed as $W_{17}$ The best correlation however was that with the fat free body weight which is an expression of the muscle mass

Schwalb & Eberl (1967) showed that adult men aged 30–50 who were only 10–20 over weight had a larger heart volume in relation to body length and that heart volume increased with weight increase This apparently also applies to boys before puberty With slight to moderate overweight there is to begin with an adaptation of the functional dimensions to the increased body weight so long as the person is able to move about sufficiently Increasing inactivity however leads to an increase in over weight while work capacity and heart volume diminish This leads to an increase in the physical effort required which results in a further increase in inactivity The vicious circle must, therefore be broken in time

## Meeting Oct 12 1968

L Kohler H E Holst Kerstin Holst Eva Mari Kohler G Sugmar & B Lindquist *Health control of 4 year-old children Preliminary results of a pilot study*

*Introduction* (B Lindquist) The aim of this study was to determine prevalence of different types of handicaps and to evaluate various parameters for screening and thereby lay the basis for the planned general health control of 4 year-old children in Sweden

Data on past and family history was obtained from questionnaires filled in by the parents As a rule these were properly filled in

The investigation comprised the following (1) Physical examination with neurological evaluation by a pediatrician (2) vision and hearing tests performed by nurses (3) dental examination by a dentist (4) mental health and social adaptation by a psychologist. The first two investigations were done on the first visit and the dental and psychological evaluations on the second and third visit respectively Items 1 – and 4 were performed at the Child Health Center and item 3 at the Public Dental Clinic

All 4 year-old children in the town of Lund in 1967 were included Of these 672 94 6 attended 20 could not be contacted and 16 families refused to allow their children to be examined Judging from other available information these children do not have a proportionately greater number of handicapping conditions

*Physical examination* (L Kohler) This examination took approximately 20 minutes and included a physical examination with neurological screening and some laboratory tests (Hgb screening for bacteriuria) Of the 636 children who participated in the health control 624 (98 ) could be completely examined In 32 of these children one or more previously unknown physical defects were detected most of them mild—e g flat foot narrow foreskin Ten per cent were referred to different specialists for further examination

Twenty four children (3 8 ) were referred to a *pediatrician* 15 because of neurological disturbances Of these five were found to have cerebral dysfunction one a chronic peripheral neuropathy one a benign central core disease

and one a gait disturbance because of a shorter leg The remaining eight children had neurological defects of no pathological significance e g a single positive Babinski with no other signs of disease One girl showed hypertrophy of the clitoris without signs of hormonal hyperactivity Of eight children referred to a urologist six girls had significant bacteriuria and one boy a persistent growth of $10^3$–10 *Proteus Mirabilis* per ml of urine and a unilateral hydronephrosis The eighth patient had a primary enuresis

Twenty children (3 2° ) were referred to an *orthopedic surgeon* for pronounced flat foot

Eleven children (1 8* ) were referred to a *pediatric surgeon* for phimosis hernia or hypospadias

Four children (0 6 ) were referred to an *otologist* Three for adenoidectomy or tonsillectomy and one for frequent nose bleeds

Only two children had less than 11 g* of hemoglobin

In screening for bacteriuria both ordinary bacteriological cultures and Uriglox® were used Uriglox® is a test paper designed to give a color reaction for the small amount of glucose normally present in urine In case of significant bacteriuria (more than 10 organism per ml of urine in two consecutive samples) the glucose is used up by the bacteria and thus the test paper gives no color reaction Out of 948 children tested six had significant bacteriuria (1 3% of the girls) none of these showed a color reaction with Uriglox® Of 942 children without significant bacteriuria 932 had a normal green color on the test paper and 10 no color (falsely positive indication) The sensitivity and specificity of Uriglox® in screening for significant bacteriuria is thus 100* and 99% respectively

*Eye examination* (G Sjögmar & L Kohler) The aim of the examination was to select children with amblyopia and squint, the two main symptoms of defective binocular vision where early detection and treatment is of utmost importance The methods of screening were (1) monocular vision examination with Boström's hooks (2) cover-uncover test in order to detect a manifest misalignment of the optic axes and (3) binocular stereopsis with the Titmus stereo test a simple method of ascertaining the presence of bifixation These methods are short (about 10 minutes) reliable inexpensive and can be performed by non professional testers

A visual acuity of 5/6 or less, a cover test suggesting squint or a defective stereo test were the criteria for referring the child to an ophthalmologist The examination could not be done in 16 children (2 5* )

Of 620 screened children 52 (8 4 •) failed one or more of the tests and were referred to the Department of Ophthalmology in Lund The examination included refraction by retinoscopy in cycloplegia In 30 cases (4 7 ) an eye disorder was present In this group 12 had simple refractive errors and one had a congenital maculopathy Of the remaining 17 (2 4 ) treatment had to be instituted immediately The main findings in this group were amblyopia in nine children and squint in eight children Except for three of the squints none of these 17 children had been diagnosed before

Of 22 children who did not pass the screening examination but subsequently showed no defects when examined in the Eye Clinic most had failed the vision test In order to reduce the falsely positive indications and thus the number of referrals the children who fail at the first examination are now retested before seen by the eye specialist

*Hearing examination* (H E Holst & L Kohler) The hearing test was performed with ordinary pure tone audiometer technique at 250 1000 and 4000 cps At 250 cps a level of 25 dB above ISO reference threshold was accepted as normal hearing at 1000 and 4000 cps the corresponding figure was 20 dB The testing was performed in a room without sound insulation The same nurse performed both vision and hearing tests, which shortened the examination period The hearing test took an average of 10 minutes

Six hundred and twenty-one children (91.6 %) were completely examined. Of these 28 (4.5 %) had a defective hearing test and were referred for further audiological examination. Twenty-one of them have been examined, in some cases after an interval of 2 months. Eight children then had a normal audiogram. Ten showed a conductive hearing loss; all but one were cured after a short period of treatment. After six months' treatment one child has a persistent abnormal audiogram and ear drum. In three children a sensoryneural lesion was found and in one it was sufficiently severe to consider use of a hearing aid.

*Odontological examination* (Kerstin Holst). The study included a dental evaluation (dental caries, gingival conditions, deposits on teeth, malocclusions as well as roentgenograms during the latter part of 1967) and an interview with the parents concerning dietary habits, oral hygiene, tooth eruption, speech development, oral habits, previous dental treatment and peroral administration and topical application of fluoride. Dietary habits were classified according to the total number of meals and snacks per day. The examination and the interview together with advice took about 45 minutes for each child.

The results were as follows: (1) 625 (93 %) of 672 children came for examination. Thirteen children (2.1 %) could not be examined. About 75 % of children examined were in need of dental treatment. The median was 4 and the maximum 5 decayed surfaces *of 88 possible* surfaces. (2) 154 (28.2 %) of 566 children were caries free. (3) Fifty-three children (9.4 %) were emergency cases and required further examination and extensive treatment. (4) Three hundred and twelve children (53 %) took 6 meals and snacks per day. Forty per cent of these children were caries-free; the median for this group was two and the maximum value 52 decayed surfaces. (5) Ninety-five children (16 %) took more than 8 meals and snacks per day. Of these 3.2 % were caries free; the median for the group was 13 and the maximum value 57 decayed surfaces. (6) Forty-eight children (8.5 %) were referred for treatment of malocclusions.

The results showed a statistically significant difference ($p < 0.001$) in the incidence of caries between children with—from an odontological point of view—good and bad dietary habits even when the teeth were exposed to cariogenic factors for only a few year. The relationship between the incidence of caries and dietary habits calls for an expanded advisory service on food and dietary habits in early age groups —e.g. at Child Health Centers.

*Examination of mental health and social adaptation* (Eva Mari Kohler). The aim was to detect and describe the mental and social disturbances of the child and his surroundings that may require advice and treatment. Information about the child and his milieu was obtained from questionnaires to both parents, observation of the child in a psychological group-examination and an interview with the mother. Where the group-examination revealed a need for further information, an individual examination was performed.

One group of children (22.4 %) had no problems, both according to the parents and the psychologist's opinion.

Every family where the father or the mother felt that the child's behavior was a problem was considered a case for treatment. In many cases (25.1 %) the examination showed, however, that the child was normally developed and well adapted. The treatment then needed was information to the parents to change their attitude to the child and his behavior.

In another group of children (38.1 %) it was necessary to discuss a change in the method of upbringing to promote the development of the child and the adaptation of the child and the family (e.g. bedwetting, irregular sleeping habits).

For children with more disturbing problems (10 %) like marked aggressiveness, overactivity and anxiety, a combination of medical, psychological and social therapy was sometimes sm

tiated—e g drugs to the child advice to the parents and kindergarten attendance

Finally a small group of children (4 4 %) needed further examination and treatment out side the Health Center mostly admittance to the hospital s pediatric or children s psychiatric clinic

*Concluding remarks* (B Lindquist) The frequency of vision and hearing abnormality was found to be about the same as could be ex pected from previous screening studies (8 4% and 4 5%, respectively) At the odontological examination a somewhat higher frequency of children in need of dental care was found than had been expected (75% ) The physical exa mination revealed, however fewer disorders than was expected (9 4% ) The reason for this is probably that the children in Lund are very well controlled The psychological examination revealed however a great proportion of 4-year old children in need of some kind of treat ment beyond general advice (14 4%)

## Meeting Nov 28–29 1968

A Dahlqvist & N Swenningsen *Galactose and lactose in urine from newborns*

Urinanalyses were performed on samples taken 2–6 days after birth from 104 normal new borns Forty three babies with neonatal icterus and 32 prematures (from some of the prema tures samples were taken repeatedly up to the age of 20 days) The samples were analysed quantitatively for galactose partly with galac tose oxidase partly with galactose dehydroge nase The reaction with a newly described galactose specific test paper has also been as certained Moreover the lactose concentration has been determined from the increase in ga lactose following incubation with a lactose pre paration Our purpose in this study was partly to compare the methods and partly to obtain quantitative data concerning the concentration of galactose and lactose in urine during the newborn period

Both methods for quantitative determination of galactose gave reliable results when tried out With both methods we found galactose in all the urine samples With galactose dehydro-genase, however somewhat lower values were found than with galactose oxidase (average value for the normal group was 20 mg% and 40 mg% respectively) The reason for this it appeared was that lactose—whenever this di-saccharide was found highly concentrated in the urine—also gave a certain reaction with galactose oxidase, but not with galactose de hydrogenase When the lactose was hydrolysed before analysis both methods for determining galactose yielded the same result

Lactose determination showed an average value of about 80 mg% in the normal group The spread was wide and some samples from healthy babies contained as much as 300–500 mg% lactose

Neither the group with neonatal icterus nor the premature group showed an increased ex cretion of galactose or lactose in the urine

L Avellán *Incidence of hypo and epispadias and exstrophy of the bladder in Sweden*

A new kind of continuous registration of mal-formations was introduced in Sweden on April 1 1964 on a voluntary basis in 39 delivery departments This system became obligatory as of January 1 1965 in 50 departments of ob-stetrics & gynaecology and maternity units with pediatric consultants Deliveries in these insti tutions represent about 60 of total deliveries in the country The incidence study was based on data reported during the period 1 4 64–30 6 67 when a total of 2677 malformations was registered for 235 500 deliveries Of these malformations 254 (9 5 %) were urogenital

Among urogenital malformations hypospadias constitutes the largest group 194 cases —that is 76 of all the urogenital cases

On the cards the hypospadias cases are registered as either isolated or associated with one or more malformations that are separate and distinct from one another Of 194 hypospadias cases 90.2 were isolated 8.3 were associated with one and 1.5 with two or more malformations

*Epispadias and exstrophy of the bladder* are rare malformations A total of 13 cases has been reported which is only 5 of all the urogenital cases Exstrophy of the bladder was reported in 7 cases—4 girls and 3 boys Among the boys only in one case was exstrophy of the bladder associated with one other malformation Among the girls exstrophy of the bladder was associated with a total of 11 very serious malformations and the mortality was 50 in the days immediately after birth

Anna Lisa Annell & Karl Henrik Gustavson *Klinefelter's syndrome in school children*

The mental characteristics of adult Klinefelter subjects are well documented mental retardation a passive-aggressive attitude mild mental disorders weak libido and emotional and social immaturity The present study was done to see whether prepubertal Klinefelter subjects also showed a high rate of mental abnormality

All the boys admitted during the last five years to the Department of Child and Youth Psychiatry in Uppsala were screened for positive X chromatin and a prevalence of 1.6 per cent was found which is eightfold that in the standard population Two of the ten subjects were of pre school age The other eight were all referred to the Department because of maladjustment in school Psychologic examination showed the same mental characteristics in all eight their intellectual subnormality psychomotoric passivity lack of endurance emotional instability and severe reading and writing difficulties Physically the boys were characterized by unusually tall stature and awkwardness The authors conclude that it is important to diagnose the syndrome as early as possible so that schooling of these subjects can be adapted to their disabilities and to protect them from the stresses associated with scholastic failure and friction between them and their classmates They also emphasize how important it is to explain to parents in what way their children are handicapped so that they accept their boys as they are and their handicap

Bengt O Eriksson Per Olsson Claes Thorén & Erik Zetterquist *Polycythemia and coagulation disorders in cyanotic congenital heart disease*

Coagulation factors were examined in 15 cases of Fallot's anomaly 7 of ventricular septal defect with pulmonary hypertension and right-left shunt 6 of transposition of the great vessels The results were correlated as regards severity and degree of desaturation the standard of measurement being the hematocrit level (hct)

No correlation was found between hct and Factor VIII Factor V specific prothrombin fibrinogen and other factors On the other hand there was a correlation between hct and thrombocytopenia and the thrombin time Increasing hct was associated with a lower thrombin level and longer thrombin time

This shows that with an increase in polycythemia there occurs a consumption of thrombocytes as well as prolongation of the thrombin time probably because of increased fibrinogen breakdown This suggests intravascular coagulation As regards the other factors there seems to be a balance between increased consumption and synthesis Thrombopoiesis seemed uninfluenced

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# PROCEEDINGS OF PÆDIATRIC SOCIETIES

## DANISH PÆDIATRIC SOCIETY

### Meeting January 15 1969

B Lautrop *An attempt to assess the prophylactic effect of whooping cough vaccine* (To be published in Ugeskrift for læger)

P E Christensen *Assessment of the side effects of diphtheria tetanus whooping cough vaccine* (To be published in Ugeskrift for læger)

*Discussion*

*Sv Heald* I have never advised against vaccination and in particular vaccination against poliomyelitis but I have emphasized verbally and in writing that tuberculosis and the so-called infectious diseases of childhood such as diphtheria, whooping cough scarlatina etc have neither been eradicated nor prevented by vaccination On the contrary I have emphasized that it is the altered conditions of life which have altered the dangerousness of these and other diseases In this opinion I am in excellent company as my ideas originate to a great extent from the internationally renowned Swedish pædiatrician Professor Arvid Wallgren, who wrote in 1956 in Nordisk Medicin (no 1 page 21) an article Is mass vaccination still required in Scandinavia? and the similarly internationally renowned epidemiologist Professor Johan Strom in Stockholm who published an article in the British Medical Journal October 1960 page 1184 Is universal vaccination against pertussis always justified?

*P Drucker* During the period 1967 and 1968 in the infant and child welfare clinic in Dronningensgade I made it a practice to question the mothers systematically about any complications or ordinary post vaccinal symptoms such as pyrexia restlessness or local reactions following triple and poliovaccination

In approximately 500 children during these two years brief periods of pyrexia had occurred in 23 (4 6 per cent) transient restlessness in 31 (6 per cent) and slight local reactions in six (1 per cent)

Among the children who developed pyrexia following the third vaccination was a female infant aged nine months and weighing 7 9 kg (birth weight 1500 g) She was admitted to hospital the same evening on account of febrile convulsions but recovered rapidly and could be discharged after four weeks in excellent health The diagnoses were febrile convulsions bronchopneumonia and otitis media

The first second and fourth (half of the normal dose) vaccinations did not result in any symptoms in the same infant

J Melchior *Infantile spasms and vaccinations* (To be published in Ugeskrift for læger)

H von Magnus *A proposed new vaccination programme* (To be published in Ugeskrift for læger)

### Meeting February 12 1969

M Yssing *Demonstration of a patient with hypercalcaemia hyperphosphataemia and peculiar skeletal changes*

A Dupont B Dupont J Bliddal E Holst J Melchior & O E Ottesen *Idiopathic infantile hypercalcaemia syndrome* (Presenta

Bjorn Bjarke, Bengt O Eriksson, Anna Lisa Holm, Sigrid Soderlund & Claes Thoren *Infants with heart disease*

Six per cent of all living children in Sweden are born with congenital heart disease—that is, about 750 children a year Of these 25–30% die during the first month of life and altogether about 35% during the first year—that is about 200 infants a year During the years 1965–30 6 1968 a total of 129 infants with congenital heart disease were treated at Kronprinsessan Lovisa s Children s Hospital in Stockholm Catheterization was performed on 55 children half of whom were less than 1 month old Of the 129 children 27% had ventricular septal defect as the main diagnosis, 15% had transposition of the great arteries, 11 % pulmonary stenosis, 9% coarctation of the aorta, and 8% so-called hypoplastic left heart syndrome 40% of the cases had more than one lesion

Total mortality in the material was 45% of which 40% died during the first week of life and a total of 60% during the first month Among those with only one lesion mortality was 23 while taking all lesions together the mortality was three times greater (76% )

Twenty four children were operated on and in most of these cases the lesion was life threatening Twelve died 9 of whom were in such poor general condition because of cardiac insufficiency and cyanosis that they could not survive an otherwise successful operation In addition to these 24 operated children, 5 atrial septostomies were carried out using a balloon catheter (Rashkind) there were no deaths An additional 12 children who died were found to have operable cardiac lesions In six of these the diagnosis was made too late, while in the other six the investigation was inadequate A total of 41 cases with extremely severe lesions were operable and this corresponds to 32% of the series

In order to reduce the high mortality among newborns because of congenital heart disease a heart investigation is necessary at an early stage as well as catheterization and angiocardiography without delay A team of experienced specialists is a prerequisite for this

*R Lagercrantz*

*Acta Pædiat Scand 58 645–652, 1969*

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*R Lagercrantz*

…ateral defect of the inter-auricular septum and a large persistent ductus arteriosus was demonstrated. Both of the internal carotid arteries were replaced by solid fibrous cords 2 cm after their origin.

Macroscopic examination of the endocrine organs revealed normal conditions.

The cranium showed moderate hydrocephalus with separation of the sutures and enlargement of the fontanelles. Fresh flame-like red haemorrhages were encountered in the membranes and a number of brownish pigmented haemorrhagic areas. The hemispheres were not developed at all but replaced by a large cyst containing approximately 300 ml xanthochromic clear cerebrospinal fluid. The falx cerebri and tentorium cerebelli were normally developed. Basally in the cranial cavity two brownish disc-like structures were encountered and were identified as the thalamic nuclei and the hippocampus, the latter being the phylogenetically oldest structure in the brain. Well-developed choroid plexus were found corresponding to the lateral ventricles and in this region there were large quantities of haematogenous pigment. Nothing remained of the occipital lobes and all that could be found of the mesencephalon were remnants of the basal part of the cerebral peduncle. The hypothalamic region was replaced by a very thin membrane of glial tissue and peripheral to this only a very fine veil of the pia-arachnoid membrane was [illegible]. The sella turcica was normal with a normal adenohypophysis, while the neurohypophysis was replaced by a fibrous scar. The olfactory bulb, the optic nerve, optic chiasma and the optic tract were not present. The eyes were normally developed with an optic nerve stump of 1½ cm in length which narrowed centrally to become a fibrous cord towards the optic foramen. From the third cranial nerve and distally the development appeared to be normal.

The intracranial part of the internal carotid artery and its branches were absent. The circle of Willis was absent but the vertebral artery and the basilar artery [illegible]

*Microscopic examination.* In the lungs basal atelectasis and bronchopneumonia were found but no evidence of cytomegalic virus inclusion disease. The suprarenals were normal. The adenohypophysis was of normal size and contained chromophile and chromophobe cells in the normal proportions. In the eye complete degeneration of the stratum ganglionare and severe fibrosis of the optic nerve were found. The brain cyst contained small islets of glial tissue, recent and previous haemorrhages in the membranes and considerable quantities of haematogenic pigment in the ependymal cells in the choroid plexus. The brain stem nuclei and the cerebellum were normally developed but the structures in the hypothalamic region could not be differentiated. Recent and old thromboses were found in the veins and the choroidal vessels.

Although the genesis of the malformation could not be deduced from these autopsy findings, they provided evidence to suggest a significant vascular factor.

Discussion

*O. Steinicke.* Have intracranial calcifications been described in the literature?

K. W. Kastrup. No.

*Henn. Andersen.* How did the endocrine organs function? Was the growth hormone response normal for the age?

*H. Søgaard.* The suprarenals were normal and the other endocrine glands were not investigated.

*K. W. Kastrup.* The growth hormone response was normal for the age.

*J. Melchior.* It is difficult to understand that brain can be resorbed. In my opinion developmental arrest was more probably present.

*K. W. Kastrup.* As mentioned previously a similar condition has been reproduced by obstruction of the internal carotid arteries in embryo puppies.

tion of the syndrome on the basis of observations in six patients)

*Discussion*

*N J Brandt* Was the excretion of amino acids and hydroxyproline investigated in these patients?

*B Dupont* No

*B Friis Hansen* Is leukoderma the same as precancerous leukoderma?

*E Holst* No

*B Friis Hansen* Does thyrocalcitonin play any part in the syndrome?

*B Dupont* That was not investigated

*E Hamberg* What is the prognosis?

*B Dupont* Many of the stenoses are probably asymptomatic and without prognostic significance If no haemodynamic symptoms are present a normal life span can probably be anticipated

## Meeting February 26 1969

K W Kastrup & E Schutzsack *Investigation of a case of hydranencephaly*

In hydranencephaly the cerebral hemispheres are reduced to thinwalled cysts The walls of the cysts consist of pia and arachnoid and are covered on the inner surface with remnants of glial tissue The condition can be diagnosed simply by transillumination The head is felt to be considerably colder than the remainder of the body A case occurring in a dizygotic twin is described The pregnancy was uneventful The mother denied having taken drugs in any form The other twin was completely healthy Infection with toxoplasmosis and cytomegalic virus could be excluded

The Moro and grasp reflexes were found to be retained Neurologically the infant remained at a stage of development between ten and twenty days of age The condition was dominated by poikilothermia and recurrent respiratory infections Terminally the condition was dominated by hydrocephalus and seizures

Evidence of diabetes insipidus with good response to the antidiuretic hormone was found Glucose and insulin tolerances were normal During the latter investigation determination of growth hormone production was undertaken and showed normal increase to hypoglycaemic response

Carotid angiography revealed bilateral aplasia of the internal carotid artery while the vertebral and basilar arteries were retained

Severe bilateral optic atrophy was present

The mechanism of development is mentioned A toxic agent probably of vascular nature has affected the already well developed brain in the last trimester of pregnancy and this has resulted in complete disintegration of the brain tissue already formed Only the structures supplied by the basilar artery were retained The condition and the pathological changes could be reproduced experimentally in embryo puppies by occlusion of the internal carotid artery with paraffin plugs

H Søgaard *Autopsy findings in hydranencephaly*

*External examination* The body was that of a little pale female infant weighing 4350 g The circumference of the head was 47 cm (normal range 38–40 cm) Distinct peripheral cyanosis was present Apart from shortening of the second and third fingers on the left hand there were no external congenital malformations

The cause of death were extensive pulmonary atelectasis stagnation of secretion in the respiratory passages and moderate basal pneumonia

Hypertrophy of the right ventricle atrophy of the left ventricle moderate valvular aortic

nenous defect of the inter auricular septum and a large persistent ductus arteriosus was demonstrated Both of the internal carotid ar teries were replaced by solid fibrous cords 2 cm after their origin

*Macroscopic examination* of the endocrine organs revealed normal conditions

The cranium showed moderate hydrocepha lus with eparation of the sutures and enlarge ment of the fontanelles Fresh flame like red haemorrha es were encountered in the mem branes and a number of brownish pigmented haemorrhagic areas The hemispheres were not developed at all but replaced by a large cyst containing approximately 300 ml xanthochro mic clear cerebrospinal fluid The falx cerebri and tentorium cerebelli were normally devel oped Basally in the cranial cavity two brown ish date like structures were encountered and were identified as the thalamic nuclei and the hippocampus the latter being the phylogeneti cally oldest structure in the brain Well-de veloped choroid plexi were found correspond ing to the lateral ventricles and in this region there were large quantities of haematogenous pigment. Nothing remained of the occipital lob s and all that could be found of the mes encephalon were remnants of the basal part of the cerebral peduncle The hypothalamic re gion was replaced by a very thin membrane of glial tissue and peripheral to this only a very fine veil of the pia arachnoid membrane was found The sella turcica was normal with a normal adenohypophysis while the neuro-hypophysis was replaced by a fibrous scar The olfactory bulb the optic nerve optic chiasma and the opti tract were not present The eyes w r normally developed with an optic nerve stump of 1½ cm in length which narrowed centrally to be come a fibrous cord towards the optic foramen From the third cranial nerve and distally the development appeared to be normal

The intracranial part of the internal carotid artery and its branches were absent. The circle of Willis was absent but the vertebral artery and the basilar arterie were normal

*Microscopic examination* In the lungs basal atelectasis and bronchopneumonia were found but no evidence of cytomegalic virus inclusion disease. The suprarenals were normal The adenohypophysis was of normal size and con tained chromophile and chromophobe cells in the normal proportions In the eye complete degeneration of the stratum ganglionare and evere fibro is of the optic nerve were found The brain-cyst contained small islets of glial tissue, recent and previous haemorrhages in the membranes and considerable quantities of haem atogenic pigment in the ependymal cells in the choriod plexus The brain stem nuclei and the cerebellum were normally developed but the structures in the hypothalamic region could not be differentiated Recent and old thrombo-ses were found in the veins and the choriodal vessels

Although the genesis of the malformation could not be deduced from these autopsy find ings they provided evidence to suggest a sig nificant vascular factor

*Discussion*

*O Steinicke* Have intracranial calcifications been described in the literature?

*K W Kastrup* No

*Henn Andersen* How did the endocrine or gans function? Was the growth hormone re-spon e normal for the age?

*H Søgaard* The suprarenals were normal and the other endocrine glands were not in vestigated

*K W Kastrup* The growth hormone response was normal for the age

*J Melchior* It i difficult to understand that brain can be re orbed In my opinion develop-mental arrest was more probably present

*K W Kastrup* As mentioned previously a similar condition has been reproduced by ob-struction of the internal carotid arteries in em bryo puppies

tion of the syndrome on the basis of observations in six patients)

*Discussion*

*N J Brandt* Was the excretion of amino acids and hydroxyproline investigated in these patients?

*B Dupont* No

*B Friis Hansen* Is leukoderma the same as precancerous leukoderma?

*E Holst* No

*B Friis Hansen* Does thyrocalcitonin play any part in the syndrome?

*B Dupont* That was not investigated

*E Wamberg* What is the prognosis?

*B Dupont* Many of the stenoses are probably asymptomatic and without prognostic significance If no haemodynamic symptoms are present a normal life span can probably be anticipated

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N Hobolth *Macrosomia A case for diagnosis*

A boy aged 2 $^1/_4$ years presented accelerated growth in length combined with absolute and relative macrocephaly The face was charac terised by a prominent forehead, broad nasal bridge large forward directed nostrils, prom inent zygomatic region a large mouth with a large cleft tongue with a very short frenulum The palatal substance was broad with a mid line depression continuing into a partially cleft uvula Closure between the oro and rhino pharynges was inadequate A systolic murmur was heard and the abdomen was prominent with a slightly enlarged liver The hands and feet were large The subcutaneous tissue was loose and scanty and the muscles were hyper plastic Air-encephalography revealed slight diffuse central cerebral atrophy Raised fasting growth hormone levels in the plasma were en countered on three occasions The clinical pic ture which was presumed to be due to a hypo thalamic defect is compared with Berardinel li s and Soto s syndromes but did not resemble either of these in all re pects The appearance and slight hypercholesterolaemia could be traced in the family so that a sex-linked dom inant heredity appeared to be present

*Discussion*

*Henn Andersen* The general consensus of opinion is that increase of growth hormone during insulin tolerance is of greater signifi cance than the fasting value In my opinion the patients look rather acromegalic Were they tall? The syndrome resembles cerebral gigan tism most of all

*N Hobolth* They were not over normal height

*J Melchior* The syndrome of increased growth in length and muscular hypertrophy is recog nised in connection with increased excretion of acid mucopolysaccharides

*N Hobolth* Increased excretion of acid muco polysaccharides was not demonstrated

*Henn Andersen* Was chromosome investiga tion undertaken

*N Hobolth* No

*C Friedrichsen* Were the mouths of the other members of the family examined?

*N Hobolth* No

*K Hauge Kristensen* In the Paediatric Depart ment in Hillerød we have seen a case of pre sumed gigantism with increased excretion of acid mucopolysaccharides

K W Kastrup *Ehlers Danlos disease*

This condition must be considered in children with hypermobile joints These may occur without accompanying changes as an inde pendent disease entity termed inter al arthro-chalasi multiplex congenita Hypermobile joints and muscular atrophy chromosome aberrations myxoedema and metabolic defects are frequently observed Ehlers Danlos disease is characterised by elastic changes in the skin with a tendency to haemorrhages and tears fol lowing minor traumata and hypermobility in the joints The mode of inheritance is auto somal dominant Milder cases may however occur in which only the skin symptoms or the joint symptoms dominate Two cases with hypermobile joints and very slight skin changes are presented In both cases kin biopsy re vealed changes corresponding to Ehlers Dan los di ease It is emphasized that the condi tion is probably more common than previously supposed and that skin biopsy with staining for elastin will frequently establish the diag nosis A primary defect is probably present in the structure of the connective tissue although it has not proved possible to reveal this by means of biochemical and electronic micro-scopical examination The defective supportive function in the tissues results in the secondary hyperplasia of the elastic tissue

*Discussion*

*C Hansted* Was the excretion of amino acids investigated?

*K W Kastrup* No The excretion of hydroxy proline was normal

## Meeting March 12 1969

E Nathan *Feeding premature infants with milk powder containing unsaturated fatty acids*

Two groups of premature babies were fed with breast milk and a cow's milk preparation (Semper Flour free Supplement) in which butter fat had been removed and replaced by fat containing at least four per cent of the calories as linoleic acid Serum cholesterol analyses were undertaken in both groups No significant differences were demonstrated

Thirty per cent of the premature infants who were fed with Semper Supplement developed slight transient irritation in the napkin region In other respects the preparation was well tolerated and the average increase in weight was greater than in the control group The composition of the preparation approximates closely the requirements which must be made of breast milk substitute particularly in respect unsaturated fatty acids Semper supplement has considered to be suitable as a substitute breast milk

[illegible]ussion

*Brestrup* The increase in weight was unusually great How old were these infants and what feeding had they previously received? It is difficult to avoid rancidity on drying in connection with the unsaturated fatty acids Was the preparation rancid?

*Hansted* What fatty acids did the preparation contain?

*Nathan* We have no information concerning which fatty acids were added This varies from time to time Nor have we any information as to whether they were added before or after drying The infants were 2–14 days of age and had previously received breast milk The increase in weight was great and cannot be explained

*Fris Hansen* Had the infants oedema?

E *Nathan* No

*P Krasilnikoff* The preparation contains more protein than breast milk Can this not explain the increase in weight?

*E Nathan* Perhaps The advantages of increase in weight are doubtful

*C Hansted* If the unsaturated fatty acids are added as triglycerides rancidity is avoided

E Nathan *Mortality in premature infants*

The results of an attempt at more intensive treatment of premature infants with the respiratory distress syndrome (RDS) are presented All patients with RDS were treated regardless of the severity of the symptoms Treatment consisted in administration of oxygen increasing to 100 per cent depending upon the clinical criteria The percentage of oxygen was increased when the respiratory rate exceeded 60 the heart action over 160 $P_{CO_2}$ over 60 and cyanosis were present Ten per cent glucose solution was administered intravenously in relatively small quantities from 30 ml per kg per 24 hours during the first two days increasing gradually to 200 ml per kg from the fifth day of life Further ampicillin was administered The patients were nursed in Trendelenburg's position and secretion was aspirated frequently from the nose and throat Sodium bicarbonate was administered according to the calculated deficit In all cases treatment was instituted immediately

The results are accounted for by registration of the mortality in premature infants and the autopsy findings for infants born in the periods 1 1–31 6 1966 1967 and 1968 The two first mentioned periods provide control materials for the present clinical trial which took place in 1968 For all patients with birth weights less than or equal to 2500 g the mortalities in the two previous periods were 19 0 and 24 8 per cent while after treatment this period fell to 13 2 per cent If the mortality is calculated

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excluding patients with birth weights of under 1000 g the figures became 17 0, 17 3 and 9 6 per cent respectively The reduction in the mortality is significant (p less than 0 001) The total number of patients admitted with birthweights of under 2500 g increased during the three periods

It was revealed that the decrea e in the mortality was particularly pronounced in the type of infants in whom autopsy revealed pulmonary changes only as the decrease here was from 14 5 to 3 5 per cent On the other hand a slight percentage increase occurred in the number of cerebral haemorrhages in the treated infants

In accounting for the mortality and the autopsy results prejudice has been avoided in the subjective assessment of which infants had RDS The significance of early treatment had been emphasized by many authors The pulmonary vasoconstriction which occurs in low oxygen tension and high $P_{CO_2}$ is also evidence in favour of early treatment

Oxygen treatment of newly born infants fell into disrepute for a period on account of retrolental fibroplasia However it has been demonstrated that the number of cases of cerebral paresis increases when the oxygen percentage is maintained under 30–40 per cent Arterial oxygen tension of under 50 mm Hg represents the limit for normal metabolic reaction in premature infants At this tension the oxygen saturation is 85 per cent in foetal haemoglobin and there is no cyanosis It is therefore indicated that sufficiently high oxygen concentration is employed immediately preferably employing measurements of arterial oxygen tension and if this is not possible, by the above mentioned clinical criteria In the material presented here no cases of retrolental fibroplasia were encountered

Early intravenous treatment was proposed by Usher who obtained a great reduction in the mortality in premature infants with this alone In the present material, considerably smaller quantities of fluid were employed The difference in the quantity of fluid required is possibly because in infants born in the County of Copenhagen relatively late ligation of the cord was undertaken

During the period in question, respirator treatment of the patients was not employed

In order to analyse the significance for the mortality of the fact that an interest had been shown for treatment of premature infants, a similar review was undertaken of the mortality in a period in which treatment with respirator sternal traction and fluid therapy had been attempted in the presence of serious symptoms During this period a slight decrease in the mortality occurred, viz from approximately 17 per cent to approximately 14 per cent (compare with the decrease obtained here from approximately 17 per cent to approximately 9 per cent)

*Discussion*

*P Brøstrup* The incidence of respiratory distress is apparently decreasing rapidly

*E Nathan* We have deliberately avoided accounting for the total number of cases of respiratory distress as the criteria are uncertain

*B Friis Hansen* How is the diagnosis of lethal cerebral haemorrhage established?

*E Nathan* All cases with macroscopic haemorrhage are included The term lethal is perhaps incorrect

*B Friis-Hansen* Were any cases of kernicterus found? I have seen several cases in premature infants at autopsy in whom the serum bilirubin had not been higher than approximately 10 mg

*J Flamand Christensen* Why was THAM (2 amino 2hydroxy methyl 1 3 propandiol) not employed?

*E Nathan* It is easier to calculate the dosage of bicarbonate

*B Friis Hansen* Necrosis of the liver has been demonstrated following the use of THAM

S Sparrevohn *Hypoglycaemia in newly born infants*

Neonatal hypoglycaemia is said to be present when blood sugar levels ≤0 20 g/l are present 3–4 hours after birth in premature infants and <0 30 g/l during the first 72 hours after birth or <0 40 g/l thereafter in full term infants

The condition is observed primarily in newly born infants with low birth weights particularly in the so-called small for dates infants with birth weight <10 percentile for the expected weight at the given gestational age In addition the condition is more frequently observed in boys the smaller of discordant twins following maternal pre eclampsia in connection with neonatal polycythaemia (i e haemoglobin values of >250 g/l) and in erythroblastosis

The incidence of neonatal hypoglycaemia is stated by the majority of authors to be about 5–6 per cent of newly born infants in departments for newly born infants

A material consisting of 228 newly born infants admitted to the Paediatric Department in the Copenhagen County Hospital in Glostrup during the period 1 1 1967–31 12 1968 are reviewed

In this material 401 infants had birth weights of ≤ 2500 g 27 had birth weights of >2500 g and 80 infants were small for dates Thirty-one per cent of the infants who were small for dates had hypoglycaemia Approximately half of these had symptoms of hypoglycaemia i e tremor jerky movements and seizures The great incidence of hypoglycaemia in the material may be due to the relatively great proportion of infants who were small for dates but the most important explanation is probably that in the blood sugar determinations with glucose-oxidase we did not take into consideration the great content of glutation in the neonatal erythrocytes which has resulted in erroneously low blood sugar values Glutation may be eliminated by addition of zinc sulphate barium or sodium hydroxide to the blood samples

*Discussion*

*Lise Wagner* How early was the first feed given?

*S Sparrevohn* In small for dates infants six hours after birth and in full term infants 12 hours after birth

*C Ingomar* How rapidly did the symptoms disappear on administration?

*S Sparrevohn* This is not known but symptoms may persist for a week

*B Friis Hansen* When was the hypoglycaemia demonstrated? How many of the patients had prolonged symptoms?

*S Sparrevohn* The first blood sample was withdrawn 2–5 hours after birth Approximately five patients had symptoms of long duration

J Christoffersen & M Egeblad *Complications of head injuries in children*

During a period of 3 1/ years 455 infants with head injuries were admitted to the Paediatric Department Copenhagen County Hospital in Glostrup Of these 382 had concussion and 43 fractures of the skull The majority of cases of concussion and fractures of the skull only required admission for 24 hours with regular control of the level of consciousness pulse and blood pressure Radiography of the cranium was undertaken in all of the patients immediately after admission If haemorrhage from the ears occurred prophylactic penicillin treatment was administered even if there had not been radiologically demonstrable fracture or liquorrhoea

As a complication a case of growing skull fracture in a male infant aged three weeks is mentioned Immediately after the trauma a palpable fracture in the right parietal region was observed and during the subsequent months this grew in width After four months therefore a polyethylene plate was introduced operatively to close the defect Apart from slightly reduced muscle power in the left arm

the patient was found to be completely healthy at follow up examination later Fifty per cent of the cases of growing skull fracture occur in infants under the age of one year and 90 per cent in children under the age of three years Rupture in the dura mater is a condition for growing skull fracture and a leptomeningeal cyst is presumed to be responsible for resorption of the bone edges Growing skull fracture is always accompanied by a slight or more severe brain lesion All children with fracture in the cranial thecae should be subjected to follow-up radiographic control every three months after the trauma

Another complication of head injury occurred in a girl aged 2 $^{1}/_{4}$ years who was admitted with slight symptoms of concussion During the subsequent ten days she was irritable and the head was held in a slightly hyperextended position Radiography of the cervical spine revealed a fracture in the odontoid process with approximately $^{1}/$ bone width s displacement Revision of the cranial radiographs from the day of admission revealed that at that time the fracture was present with displacement of 2 mm The patient was treated with a Glisson s sling for three months and became symptom free The case is reported to emphasize the fact that in ordinary radiographs of the skull the upper part of the cervical spine can always be seen and it is therefore possible to discover any fractures there

Niels Michelsen *Sjogren–Larsson s syndrome*

In 1957 Sjogren & Larsson described 28 individuals with an autosomal recessive hereditary syndrome the cardinal symptoms of which were 1) congenital ichthyosis 2) cerebral paresis of spastic type and 3) oligophrenia Three of the patients had in addition degeneration of the pigmented epithelium in the macula and surroundings with resulting deprivation symptoms

Since then, many similar cases have been described from many countries Three Danish cases are reported, all of whom had characteristic symptoms Review of the literature and investigation of the author s own cases did not reveal any definite explanation for the pathogenesis In particular no definite metabolic, hormonal or chromosomal changes could be found

Erling Nathan & Jens Christoffersen *Iatrogenic hydrothorax Report of a case in a female infant aged nine days*

In rare cases hydrothorax has been described as a late complication of infusion via a venous catheter Five out of the eight published cases are children No cases in children have previously been published from Denmark

The present patient was admitted to hospital on account of vomiting On the day after admission a venous catheter was introduced into the right cephalic vein Twelve hours later during radiography the patient became acutely ill Intubation and ventilation were required Radiography in Trendelenburg s and horizontal positions revealed a displacable density on the right side Pleural puncture was undertaken and 132 ml slightly turbid fluid was withdrawn This contained so much glucose that only infusion fluid could be concerned After removal of the catheter the patient recovered completely Analysis of the patient s serum and of the pleural fluid showed decrease and increase respectively in the electrolyte content as evidence of dialytic effect

The reason that this complication apparently occurs more frequently in children than in adults may be that children are more motorically restless and that their thoracic movements particularly during screaming are greater and that their veins are more thin walled The risks involved in employment of stiff or possibly pointed catheters are emphasized

*P Paerregaard*

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# PROCEEDINGS OF PAEDIATRIC SOCIETIES

## THE FINNISH PAEDIATRIC SOCIETY

### Meeting February 22 1969

! Karlberg (Gothenburg) *The functional ad aptation of the neonate to extrauterine life*

### Meeting April 12 1969

J F Forfar (Edinburgh) *Some aspects of neonatal bacterial infection*

Problems related with prevention of hospital infections in neonatal wards were presented

### Meeting May 7 1969

J M Tanner (London) *The diagnosis and treatment of dwarfed children*

In evaluating growth high accuracy in the measurement of height is a necessity The measurements should be reproducible to ±0 3 cm This means that the measuring must be done by one trained person equipped with proper anthropometric instruments Every major pediatric centre should have a small growth laboratory

When the effect of a treatment on growth velocity is assessed the minimum period of observation is one year because every child has seasonal fluctuation in the velocity and that may be quite marked

Recording actual velocity of growth will reveal an acceleration much more readily than recording the growth attained as is most often done

The normal curves of attained height as well as those of velocity when based on cross sectional (one time) study of a large number of children are incorrect in that the adolescent growth spurt is flattened out as it occurs at varying ages Such curves are the most useful ones however when the height of a child who is seen only once has to be evaluated In following a child s growth curves based on longitudinal study of normal children are preferable In these curves the adolescent growth spurts of the individual children have been made to concur and the curves thus show the adolescent growth spurt as it occurs in the individual

The pituitary glands from some 25 000 autopsies yearly are collected in Great Britain and thereby about 80 children can be treated with human growth hormone (HGH) This work is directed by the Medical Research Council

According to present experience children with HGH deficiency will have a catch up growth spurt when the treatment is started As in other situations of catch up growth the velocity will slow done as the normal height range i approached It seems that the patients can be made to reach their genetically determined stature (provided HGH antibodies are not formed) but not beyond it HGH antibodies have occurred in 4 out of the 50 children treated thus far

*E I Wallgren*

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# PROCEEDINGS OF PAEDIATRIC SOCIETIES

## THE EUROPEAN SOCIETY FOR PAEDIATRIC ENDOCRINOLOGY

*Abstracts of papers read at the Eighth Annual Meeting*
*Malmö Sweden June 26–27 1969*

Jørgen Pedersen (Royal Maternity Department Rigshospitalet Copenhagen intr by C G Bergstrand) *The child of the diabetic mother Present status of the hyperglycaemia-hyperinsulinism theory*

The old and simple theory may be stated as follows Maternal hyperglycaemia results in foetal hyperglycaemia and hence in hypertrophy of foetal islet tissue with insulin hypersecretion This hyperinsulinism in the presence of more than adequate supplies of glucose abruptly eliminated at birth will explain several of the characteristic features observed in the off spring Based upon investigation performed by the author and his co workers (in particular Drs Mølsted Pedersen Wagner Klebe Hagen and Osler) and on those in the literature the morphological and biochemial evidence of hyperinsulinism in foetus and infant will be discussed The presentation will mention studies of islet cell mass interstitial cellular infiltrates in the islets and karyometric studies of the beta cell nuclei as well as insulin (IRI) glucose (*k* values) and FFA concentrations during the neonatal period Particularly I shall stress the close conformity of the results as regards the relation to birth weight and timing during the neonatal period In the second part direct and indirect evidence for the conclusion that maternal hyperglycaemia is a main factor in explaining the foetal hyperinsulinism will be put forward The third part starts with a survey of the characteristics of the off spring of diabetic women which may be described at present under four headings Anatomy Functional capacity, congenital malformations and Perinatal mortality It is concluded that the population is inhomogenous as regards birth weight and that the anatomical picture and the functional capacity display an inharmonic picture The birth weights of infants of diabetic mothers are considered in the light of the regression of *k* values on birth weight The glycaemia insulin factor may be considered as one of several common growth factors for the foetus, but although infants of diabetic women are bigger than normal they are only half as big as might be expected according to their hyperinsulinism These infants may lack a corresponding or matching oversupply of say growth hormone or display additional inconsistencies of the metabolism of growth

Bengt Persson (Kronprinsessan Lovisas Barnsjukhus Pediatriska kliniken Karolinska Institutet Stockholm intr by C G Bergstrand) *Lipid metabolism in newborns of insulin dependent and gestational diabetic mothers*

Normal infants how a rapid postnatal rise in plasma free fatty acids (FFA) In contrast the FFA values are low in offsprings of diabetic mothers An enhanced lipid mobilization in normal newborns is further evidenced by simultaneous increase in plasma glycerol and the subsequent rise in blood ketones and triglycer

des In adults lipid mobilization is influenced by nutritional neurogenic and hormonal factors and as recently demonstrated the activation of adipose tissue lipase by lipolytic hormones is mediated by 3 5 cyclic AMP The precise mechanism behind the fat mobilization in the newborn is not well understood Some factors that could influence this process such as pre and postnatal asphyxia blood levels of lactate β hydroxy butyrate glucose and environmental temperature were studied in newborns of non-diabetic mothers None of these factors seemed to influence lipid mobilization as reflected by the increase in FFA and glycerol but there was a great variation in individual values

During the last 3 to 8 weeks of pregnancy observations were made on maternal blood glucose (5/day) foetal heart rate and urinary excretion of oestriol in insulin dependant and gestational diabetics ($k<1.0$) Irrespective of the achieved degree of diabetic control the infants had low FFA levels during the first hours of life In contrast there was a marked rise in glycerol These changes were unrelated to blood glucose levels in the infants

The results are not likely explained by a basic difference in lipolysis between normal newborns and offsprings of diabetics The lower FFA levels in the latter group might indicate a suppression of the FFA release as a result of an increased rate of re esterification This hypothesis implies an increased availability of glycerol 3 phosphate which could be supplied by an accelerated entry of glucose into adipose tissue Assuming that the process of lipolysis is not as insulin sensitive as that of re-esterification this hypothesis could be consistent with a state of functional hyperinsulinism Alternatively glycerol 3 phosphate could be formed from breakdown of glycogen within adipose tissue

G Sterky B Persson & B Strandvik (Kronprinsessan Lovisas Barnsjukhus Pediatriska Klinik— Institutet Stockholm intr by C G Bergstrand) *Intravenous glucose tolerance in overweight newborns*

The means available to identify women during the preclinical phase of diabetes is still controversial Mothers of overweight newborns (>4 5 kg at term) are said to enter the high risk group Unfortunately various glucose tolerance tests performed on the mothers after delivery do not seem to reveal the diabetic state

In the present study 129 intravenous glucose tolerance test were performed in overweight newborns during the first days of life We suggest that those 35 overweight newborns (23 ) who show an intravenous glucose tolerance test similar to that of offsprings of mothers with overt or gestational diabetes are the result of an abnormal gestation of diabetic nature

Mothers of such infants ought thus to be specially taken care of and perhaps treated during forthcoming pregnancies Such a performance would possibly reduce foetal loss and could eventually reduce the risk of diabetic manifestation As in other studies of pre-clinical diabetes the final answer lies in the results of follow up examinations

Johan Gentz (Kronprinsessan Lovisas Barnsjukhus Pediatriska Kliniken Karolinska Institutet Stockholm intr by C G Bergstrand) *Transient diabetes of the newborn*

This diabetic syndrome has been described in 30 infants It is most likely to occur in infants less than six weeks of age who at birth are underweight for their period of gestation Presenting symptoms are usually a failure to thrive and sudden severe dehydration associated with polyuria and fever in the absence of diarrhoea and vomiting There is marked glucosuria and the hyperglycemia usually exceeds 600 mg/100 ml Ketonuria if present is mild The syndrome has many features in common with hyperglycemia hyperosmolar coma

The etiology of the syndrome of transient diabetes of the newborn remains unknown

A failure of insulin production due to lack of stimulation because of a low blood sugar con centration in the mother has been suggested This hypothesis seems unlikely Two cases are presented who started with symptomatic hypo glycemia at 12 and 6 hours of age and at 7 and 5 days of age respectively developed marked hyperglycemia Both infants were treated with 1 to 8 units of regular insulin per day for ap proximately 6 weeks In one of the infants intravenous glucose tolerance tests were per formed at 6 hours and 55 days of age Both times the clearance rates were very fast ($k_G$ 4 6 and 5 7 respectively) with insulin responses comparable to those seen in normal babies During the period of hyperglycemia before in-sulin treatment the plasma insulin values (radio immunoassay) were 6 and 17 $\mu$U/ml with blood sugar of 1280 and 2300/100 ml respectively Simultaneously the FFA values were 0 77 and 1 25 mmol/l

When last seen at 13 and 7 months of age respectively both infants appeared completely normal

R François A Ruiton Ughengo & J J Picaud (Hopital Edouard Herriot Lyon) *Hypoglycemia and hyperinsulinemia in newborns of diabetic mothers*

51 newborns of diabetic mothers were hos-pitalised in the department 23 of them had for one or several days a low blood sugar un der 30 mg/100 ml (determined with Somogyi Nelson method twice a day for ten days)

Five of 23 only exhibited clinical manifesta tions convulsions disappearing after i v injec tion of hypertonic solutions of glucose No manifestations of hyperadrenalinism was ob served

One patient, without clinical signs had a low blood sugar under 10 mg/100 ml for 2 days In this child encephalopathy was ob-served a few years later without other explana tion but the hypoglycemia in the neonatal pe riod

Hypoglycemia was more important in in fants whose mother had an instable diabetes without good control

Insulin was determined by radio immuno-assay (Hales Randle method) in 15 of these newborns We did not check the presence of antibodies against insulin in the plasma 14 times/15 the level of insulin was found statis tically higher than normal (above +2 S D ) Highest levels were observed in infants with the lowest blood sugars and the most severe clinical manifestations

Insulin was also determined in 11 of these newborns when they were 2 to 9 years old under stimulation with glucose per os 30 g/m of body surface In 9 children there was a nor mal increase of the insulin level from 7 micro units/ml to 40 micro-units/ml 30 min after the glucose intake In 2 of these children (one of them was born of parents both diabetic the diabetes of the mother of the other one was not well controlled during pregnancy)

An abnormal increase of the insulin level was observed (above 100 micro units/ml at 30 min)

These two children 2 and 4 years old were very tall (more than 2 S D ) The hypoglycemia curves were of paradiabetic type in the first child and showed a post stimulative hypoglyce mia in the second one

We think these two children present some risk to become diabetic The methods of pre vention are very poor meals smaller and more frequent along the day for avoiding an exces sive stimulation of Langerhans islets

J Girard M Vest & J B Baumann (Univer sity Children s Hospital Basel) *Growth hor mone in blood and in cerebrospinal fluid*

Growth hormone has been measured in a series of children under insulin induced hypoglyce mia It was found that a normal growth hor mone response can be expected if the value before injection of insulin exceeds 4 ng/ml

A single determination under basal condi

tion is therefore recommended as a screening test for growth hormone deficiency before performing a provocative test

In a small series of children growth hormone has been measured simultaneously in capillary blood and in cerebrospinal fluid. Growth hormone can be detected in cerebrospinal fluid the concentration is however not related to the plasma level measured at the same time

plasma G H in all cases ranging from 10 to 50 ng/ml

These findings confirm that there is a direct interplay between the gonadal hormones and the pituitary G H releasing system and suggest that the secretory pattern of G H is dependant on the concentration of circulating gonadal hormones. Gonadal hormones may promote linear growth by increasing the production of pituitary growth hormone

H W Rayner & G A Brown (Institute of Child Health University of Birmingham intr B T Rudd) *Growth hormone production during puberty in hypopituitarism*

The pubertal growth spurt has in the past been attributed to gonadal and adrenal androgens and oestrogens. Although it is recognised that the pituitary output of growth hormone (G H) to known provocative stimulation is increased during puberty the relative importance of GH and sex hormones in linear growth promotion remains undecided. Several workers have demonstrated that testosterone administration promotes an increased G H production in sexually retarded males and pretreatment with oestrogen is known to enhance the pituitary G H response to arginine infusion

We have assessed G H production following pituitary stimulation in five patients with hypopituitarism (four males and one female) both before and during puberty. In three cases the aetiology of the pituitary defect was unknown one followed head injury and one case had a chromophobe adenoma. In three cases an isolated deficiency of G H was present and in two it was combined with thyrotrophin deficiency

When assessed prepubertally between 10 and 13½ years all were growing at least 3 0 cm/year and all produced levels of plasma GH measured by radioimmunoassay of less than 9 ng/ml. Puberty occurred spontaneously with accelerated rates of linear growth ranging from 5 to 9 cms/year. Further assessment during puberty demonstrated increased levels of

J C Job J Lambertz & P C Sizonenko (Hopital Saint Vincent de Paul Paris intr by C G Bergstrand) *Growth and serum growth hormone in children with craniopharyngioma*

Growth velocity in 8 children presenting with growth failure which led to the diagnosis of craniopharyngioma varied from 0 to 2 2 cm/6 months without any treatment

Postoperative growth velocity has been evaluated in 22 children after removal of a craniopharyngioma. The patients with overt TSH deficiency received daily 5 to 10 cg of dessicated thyroid powder. Some patients received hydrocortisone. None received HGH or anabolic steroids. Growth velocity varied from 0 to 5 5 cm/6 months. Postoperative growth spurts of 3 5 to 5 5 cm/6 months were observed in 6 children mainly in the first 6 months after surgery but continued 2 years in 2 patients. Patients receiving hydrocortisone at doses of more than 0 3 mg/kg/day were among those with smallest height increases and in 2 of them a growth spurt was observed *after reduction of hydrocortisone*

Serum growth hormone has been evaluated by radioimmunoassay after arginine and/or insulin provocative tests. Preoperatively of 8 patients studied (8 with insulin 4 with arginine) 2 only had detectable values of serum GH none responded to insulin 1 had a weak response to arginine 1 had normal response to arginine but grew only 2 cm in one year. Postoperatively of 17 patients studied (17 with insulin 6 with arginine) none had normal GH

values, 1 had a borderline response to both tests, 5 had low but detectable amount of GH at a time No definite correlation was found between postoperative serum GH and growth velocity patients without detectable GH grew 0 5 to 3 5 cm/6 months

It must be concluded that after removal of a craniopharyngioma growth may be normal at a time without evidence of GH secretion But insulin and arginine stimulation evaluate pituitary reserve of GH and not secretion of this hormone Therefore further work will be necessary to study this problem

O Westphal (Department of Pediatrics University Hospital Uppsala intr by C G Bergstrand) *Human growth hormone (HGH) production during exchange transfusion*

An attempt is made to study the HGH production during exchange transfusion in 14 infants aged 2–5 days the birth weights varying between 1800 and 4130 g The indication for the exchange transfusion was hyperbilirubinaemia in 5 cases due to rhesus incompatibility in 3 due to ABO incompatibility while there was no incompatibility in 6 cases Donor blood preserved with glucose and disodium citrate was used glucose concentration in donor blood varied between 350 and 400 mg %

For the study of the HGH production a computor programme with the following assumption was composed

1 Half time of HGH 14 min
2 The outflow of HGH was proportional to the HGH concentration
3 No significant backflow of HGH from the extraplasmatic pool
4 No mathematic formula was calculated but every exchange transfusion was simulated by the computor

*Results* The estimated HGH levels decreased slightly in most infants during the first 10 min and increased in the following 30 min Then there was a distinct difference between infants with rhesus incompatibility and other infant the former group showing a further increase HGH levels the latter a steady state or d crease during the rest of the exchange tran fusion

The initial as well as the maximal HGH pro duction was highest in the infants with rhesu incompatibility There was a very good corre lation between maximal production and initi production Calculated total production durin the exchange transfusions varied between 1 and 550 microg totally and between 5 an 12 microg when calculated per kg bod weight There were minor differences betwee the exchange transfusions according to age an prematurity

H Zachmann & A Prader (Department c Paediatrics, University of Zurich) *The ana bolic and androgenic effect of testosterone i sexually immature boys and its dependency o growth hormone*

The effect of a long acting testosterone pre paration (Triolandren®) on growth, on skeleta maturation and on secondary sex characteris tics was studied in 24 boys with sexual im maturity of different causes In patients with out growth hormone deficiency dosages above 100 mg/m /month lead to a maximum growth velocity already during the first 6 months o treatment Smaller initial dosages followed by a gradual increase lead to an imitation of the normal pubertal growth spurt The time from the start of treatment to the development of axillary hair is inversely proportional to the mean dosage In patients with GH deficiency the response is less marked with respect to growth and to the development of secondary sex characteristics To exert its full growth promoting anabolic effect testosterone appar ently needs the presence of GH To exert its full androgenic effect on the secondary sex characteristics it also needs the presence of pi tuitary hormones but it is not clear whether this effect depends on GH or ACTH or on both

J L Van den Brande J J Van Wyk F S French A L Strickland & W B Radcliffe (Depts of Pediatrics and Radiology University of North Carolina and Dept of Pediatrics School of Medicine Rotterdam intr by H K A Visser) *Effect of thyroid hormone and cortisone on the growth and skeletal maturation of hypopituitary children and its modification by human growth hormone treatment*

Nine children with documented pituitary dwarfism were studied on three regimens No treatment treatment with thyroid hormone and cortisone and treatment with thyroid hormone cortisone and growth hormone Average length of observation was respectively $3\ ^{1}/_{1}$ yrs $1\ ^{10}/_{12}$ yrs and $1\ ^{9}/_{1}$ yrs

From the increments in height age ($\Delta$HA) skeletal age ($\Delta$BA) and chronological age ($\Delta$CA) the ratios $\Delta HA/\Delta CA$ and $\Delta BA/\Delta CA$ were computed

*Results*

| | Mean $\Delta HA/\Delta CA \pm s$ | $\Delta BA/\Delta CA \pm s$ |
|---|---|---|
| No treatment | $0\ 53 \pm 0\ 19$ | $0\ 47 \pm 0\ 32$ |
| Thyroid hormone and cortisone | $0\ 65 \pm 0\ 09$ – | $-1\ 41 \pm 0\ 32$ |
| Thyroid hormone cortisone and human growth hormone | $1\ 24 \pm 0\ 48$ | $1\ 38 \pm 0\ 33$ |

$-0\ 001 < p < 0\ 01$
$0\ 01 < p\ \ 0\ 02$ (Student *t* test)

The data suggest that thyroid hormone and cortisone if not balanced by the simultaneous presence of growth hormone may adversely affect the final stature by unduly stimulating bone maturation without significantly affecting the growth rate

S S Najjar (Department of Pediatrics American University of Beirut, Beirut intr by A Prader) *Primary dwarfism with elevated serum HGH levels*

Two siblings a $13\,^{1}/_{-}$-year-old girl and a $9\,^{1}/_{-}$-year-old boy products of a consanguineous marriage had severe short stature clinically indistinguishable from pituitary dwarfism *Their basal levels of serum* immunoreactive growth hormone were persistently elevated and varied from 10 to 25 m$\mu$g/ml in the girl and from 17 to 50 m$\mu$g/ml in the boy Dilution curves of the sera were parallel to the standard HGH curve on radioimmunoassay Glucose administration orally and intravenously resulted in a *paradoxical release of growth hormone* Arginine monochloride infusion insulin hypoglycemia and Bovril administration further increased the serum HGH levels There was no significant increase in the serum insulin levels following glucose administration The *basal levels of FFA were elevated* they increased further on prolonged fasting and they failed to suppress promptly after glucose administration

HGH administration in doses of 2 and 4 mg daily for five consecutive days each failed to produce nitrogen retention hypercalciuria increased urinary hydroxyproline excretion and decrease in blood urea nitrogen

E Joss (Universitäts Kinderklinik Bern) *Genetic dominant transmitted dwarfism subresponsiveness to growth hormone?*

A family is described in which *dominant transmitted dwarfism* could be traced back for 6 generations The dwarfed members of the family reached an adult height of about 140 cm the sexual development was retarded but normal

The propositus a $10\,^{7}/_{1}$ year old boy in good health had anthropometric data comparable to those of a GH-deficient dwarf Height 112 cm ($-6\ 1$ S D below the mean) height age $5\,^{7}/_{1}$ year growth rate 2 7 cm/year bone age (Tanner) $6\,^{4}/_{1}$ year (60 of chronological age) Routine endocrine examination and skeletal survey was normal except the marked retardation of the skeletal maturation

*Measurements of immunoreactive GH* were normal Insulin induced hypoglycemia led to a prompt rise to 10 6 ng/ml and on a i v glucose load a late rise of plasma GH was noted

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*Measurements of immunoreactive GH* were normal Insulin induced hypoglycemia led to a prompt rise to 10 6 ng/ml and on a i v glu cose load a late rise of plasma GH was noted.

*The metabolic effect of exogenous HGH* compared with that of 5 GH deficient dwarfs and 3 primordial dwarfs ( small normal ') was even smaller than that, observed in the primordial dwarfs

1 *Nitrogen retention* on HGH 2 mg/m / day was only 4 mg/kg/day on 4 mg/m /day however 44 mg/kg/day No decrease of BUN was measured in contrast to GH deficient and primordial dwarfs

2 *Treatment with HGH* (Raben) 10 mg/m²/week for 6 months led to a insignificant increase of the *growth rate* from 2 7 to 3 4 cm/year which gives a difference of only 0 7 cm/year The 3 primordial dwarfs had an increase in growth rate of 1 3–1 9 cm/year

3 Treatment with HGH 4 mg/m /day for 6 days had no effect on *insulin secretion* measured under the stimulus of a i v glucose load Under the same dose of HGH Rimoin found an increase in insulin secretion in normal subjects and in GH deficient dwarfs but not in the African pygmies

Subresponsiveness to growth hormone may be suspected in our patient with dominant transmitted dwarfism but is difficult to prove as primordial dwarfs and normal subjects also show a small metabolic response to HGH

D Schonberg & J R Bierich (Universitats Kinderklinik Tubingen) *Clinical evaluation and pituitary function in familial dwarfism*

9 out of 13 children of 4 families showed typical symptoms of hypopituitary dwarfism Parents were of normal height and not related to each other Pituitary function was evaluated by the following investigations insulin tolerance test GH release thyroidal ¹³¹I uptake achillophotomotogram, plasma cholesterol 17 OHCS in plasma and urine before and after ACTH Metopirone test, urinary 17 KS urinary gonadotrophin vaginal smears

| | GP | RP | RK | FK | IB | KG | EG |
|---|---|---|---|---|---|---|---|
| GH release | decr | / | / | decr | decr | / | / |
| Corticotroph function | incr | / | incr | incr | n | n | n |
| Thyrotrophic function | decr | decr | n | n | decr | decr | decr |
| Gonadotroph function | decr | / | subn | subn | decr | decr | decr |
| Onset of growth retardation | 5 y | 3 5y | 0 5y | 0 8y | 8y | 10y | 6y |

The 2 brothers of the 4th family refused a thorough investigation in addition to somatotrophic deficiency they exhibited a markedly retarderd sexual development The degree of pituitary dysfunction was strikingly similar between siblings but varied considerably between families In G P and F K glucose response to insulin was normal in spite of absent increase of plasma GH Apparently the enhanced stimulation of the pituitary adrenal axis which could be demonstrated served as a compensatory mechanism The gross obesity of all patients of families P and K may be due to the same adrenal overactivity

The genetically fixed pattern of pituitary dysfunctions in certain families is emphasized

O Butenandt (Universitats Kinderklinik Munchen intr by D Knorr) *The influence of human growth hormone on enzymes in the blood of hypopituitary patients*

Several serum and erythrocytic enzymes were measured in dwarfed children during shortterm application of HGH over 5 days (1) and during *long term treatment over 4 weeks* Whereas alkaline phosphatase may show insignificant fluctuations during shortterm there is a significant rise during longterm treatment The leucinaminopeptidase increases during short and longtermtreatment creatinphosphokinase slightly during longtermtreatment Other enzymes did not change

A constant rise was found in glucose 6-phosphatdehydrogenase in erythrocytes in all but two children during shortterm application of HGH Pyruvatkinase and glutathionreductase did not show constant changes

Increased activity of serum enzymes can be the result of increased *production of enzymes*, an increased permeability of the cellwalls or a destruction of the latter or a decreased clear

ance of enzymes from serum Possible explanations for the activity changes due to HGH are higher metabolism of the cells of origin as it is believed to be the case in alkaline phosphatase or changed cellwall permeability on the base of altered cell metabolism (2)

The increase of enzyme activity within erythrocytes demonstrates possibly an increased formation of enzyme protein due to the anabolic effect of HGH But since especially G 6-PDH activity is higher in reticulocytes than in older erythrocytes one can assume that the enzyme increase is due to an intensified erythropoesis However no changes of reticulocyte or erythrocyte count paralleled the increased G 6 PDH activity in our investigations

1 Prader A Illig R Szeky J & Wagner H Arch Dis Childh 39 535 1964
2 Sebastandt, O Israel J Med Sci 4 785 1968

R Sterdijk (Department of Paediatrics University of Amsterdam) *Growth in firstborn and laterborn infants*

In a longitudinal study on growth during the first year of life (30–360 days) the *average* length weight and skull circumference at 30-day intervals were calculated for each of the following 4 group *59 firstborn boys 68 later* born boys *53 firstborn girls and 70* laterborn girls

It appeared that in each sex and for each of the 3 measurements firstborn children grew faster at a fairly even rate *than laterborn children* The trend of the *differences in* length weight and skull circumference *of first* and laterborn children at the *consecutive 30 day* intervals was highly significant ($p < 0.005$)

For all measurements except skull circumference in girls firstborn children *were smaller* than laterborn children at 30 days and larger at 360 days *Skull circumference in firstborn* was always larger The differences in these average values were *not significant* ($p > 0.05$)

In a number of studies *from* the literature it has been found that *at birth* firstborn children on the average are smaller than laterborn children whereas some years later firstborn children are larger than laterborn children The present study yields additional information since it shows that throughout most of the first year of life firstborn children grow faster than laterborn children

Edna H Sobel & Hilda K Bettmann (Albert Einstein College of Medicine Bronx N Y)
*Oxandrolone in the treatment of short stature*

Twenty one boys and six girls aged *4 to 18* years were treated with Oxandrolone during the second 6 months of an 18 month period Measurements of length and skeletal age were made at the beginning and at 6-month interval The daily dose was 0 05 mg/kg (6 patients) 0 1 mg/kg (19 patients) or 0 20 mg/kg (7 patients) The results were evaluated in terms of increments in height age and skeletal age Predictions for adult height at the beginning of the study were compared with those at the end of the treatment period and at 6 months to 4 years after termination of treatment

*Results* 1) Responses were similar on all three doses 2) In all but 3 children the rate of increase in height age was significantly increased during treatment this increased rate *persisted* in the subsequent 6 months 3) Increments in *skeletal* age showed no statistically evident difference attributable to treatment but was significantly increased in the 6 months following treatment Five children had an excessive advance of bone age and decrease of predicted height ranging from 2 5 to 7 1 centimeters *There* was no correlation between the ratio of *skeletal* age to height age at the onset and the *relative* rates of increase in skeletal age and height age during treatment No *patient of* adolescent age showed an excess *advance of* bone age or loss of predicted mature *height* 4) When Oxandrolone treatment coincided with the adolescent growth spurt there was a temporary additive effect on velocity but no *effect* on the long term adult height prediction

Supported in part by a grant from the G D Searle Company Chicago, Ill

B Weber W Hirsch & K Shibata (Universitäts Kinderklinik Berlin intr by E Werner)
*Cerebral gigantism hormonal studies and dermatoglyphic patterns*

Somatic acceleration sometimes present at birth and existing throughout childhood associated with clumsiness acromegalic features retarded psychomotor development and evidence of nonprogressive cerebral dysfunction has been named cerebral gigantism since Sotos *et al* (1) in 1964 described the characteristic symptoms of this disorder As it has so far only been diagnosed in children prognostic statements concerning the further development can hardly be made The aetiology of cerebral gigantism is unknown Intracranial lesions apart from hydrocephalus have not been reported Hormonal dysfunction does not seem to exist Chromosomal studies showed normal karyotypes The existence of similar relatively rare dermatoglyphic patterns in some of the children (2) however suggests either an early prenatal cerebral damage or a genetic aberration In 1968 we had the occasion to observe two girls aged 1 ½ and 2 ½ years in whom cerebral gigantism was suspected Overgrowth and massive stature strong muscular development, accelerated bone maturation and retarded psychomotor functions were found in both On one case (C W) the mother in the other (N F) the father have likewise been exceptionally tall throughout childhood Studies of carbohydrate metabolism and the hormonal function of the hypophyseo adrenal axis the thyroid gland and the pituitary (STH secretion) revealed no significant abnormalities in either of the children Precocious puberty was excluded The lean body mass was very large in both No chromosomal aberrations were found In C W on one hand in N F on both dermatoglyphic patterns similar to those reported in the literature were registered The father of N F whose childhood development according to his own statement, was similar to that of his daughter demonstrated the same rare dermatoglyphic variations Although many of the symptoms encountered in cerebral gigantism may represent normal variations within the upper range of the respective scales the accumulation of these symptoms in one individual seems to suggest the existence of a rare disorder which may be genetically determined

1 Sotos J F *et al* *New Eng J Med* 271 109 1964
2 Milunsky A *et al* *Pediatrics* 40 395 1967

S Raiti, C Light & R M Blizzard (The Johns Hopkins Hospital Baltimore and Institute of Child Health London intr by C G Bergstrand)
*F S H levels (radioimmunological) in the urine serum of boys and serum of boys and adult males*

F S H was measured (1 2) in the serum and urine of all males studied (age 5 to adulthood) Significant rises were seen at 12–13 years and at stage 3 of sexual development (mean age = 13 1 ± 0 7 years)

The 24 hours urinary excretions were 2 0 ± 1 1 2 7 ± 1 2 5 7 ± 2 8 7 2 ± 4 2 7 8 ± 3 8 and 8 6 ± 3 6 and 8 6 ± 3 6 I U of 2nd I R P H M G in the 5–8 9–11 12–13 14–15 16–18 year and adult groups The same values were 2 2 ± 1 1 4 4 ± 2 5 7 1 ± 3 0 7 8 ± 5 5 6 9 ± 3 5 for stages 1 2 3 4 and 5 of sexual development

The serum values were 4 2 ± 0 7 5 2 ± 1 1 5 8 ± 2 9 8 0 ± 3 6 7 5 ± 1 9 8 5 ± 3 6 and 7 9 ± 1 9 m IU/ml of 2nd I R P H M G for the 5–8 9–11 12 13 14–15 16–18 year and adult groups These same values were 4 5 ± 0 9 5 9 ± 1 4 8 1 ± 3 0 8 5 ± 3 2 and 7 2 ± 2 2 m IU/ml for stages 1 2 3 4 and 5 of sexual development

We conclude that FSH is found in the serum and urine of children from a very early age that the levels rise with the onset of puberty and that the FSH values after stage 3 of sexual

developm nt or 13 years of age are not signifi cantly different from those of adult males

1 Midgley A R Jr J Clin Endocr 27 295 1967
2 Raiti S & Blizzard R M J Clin Endocr 28 1719 1968

5 Almqvist R Ekholm P Olin & G Vecchio (Crownprince s Lovisa s Children Hospital Stockholm intr by C G Bergstrand) *Human fetal thyroglobulin-correlation between the biosynthesis of thyroglobulin and the ultrastructure of the fetal thyroid gland*

The human fetal thyroid gland starts to concentrate iodide and to form thyroid hormones when the fetus is 65 mm in crown rump length (CR) (1) Little is known about the time of appearance of 19 S thyroglobulin in fetal life

The thyroids from human fetuses were labelled *in vitro* with $^{125}$I and $^{3}$H leucine The soluble proteins were analyzed by sucrose gradient centrifugation The glands were also studied by electron microscopy

In fetuses of 28–55 mm CR the thyroid did not incorporate $^{125}$I Only 3–8 S proteins were labelled with $^{3}$H leucine The ultrastructure of the thyroid from a 47 mm CR fetus showed irregular cords of cells but no follicules containing colloid The cytoplasm of the thyroid cells did not contain any developed endoplasmatic reticulum The ribosomes were free

Thyroglobulin labelled with $^{125}$I and $^{3}$H leucine was demonstrated in the thyroid from fetuses of CR 65–200 mm

$^{125}$I labelled fetal thyroglobulin was identical to adult thyroglobulin according to sedimentation properties in sucrose density gradient centrifugation immunological reactivity with anti human adult thyroglobulin antiserum and the presence of iodinated aminoacids including thyroxine The ultrastructure of the thyroid gland from fetuses more than CR 65 mm corresponded to that of the adult gland The thyroid contained follicules filled with colloid The cytoplasm of the follicular cells was filled with rough surfaced endoplasmatic reticulum

In on [illegible] n the thyroid could not incorporate $^{125}$I However a small but distinct $^{3}$H leucine label appeared in the 19 S proteins This gland did not contain any follicules However the cytoplasma was rich in rough surfaced endoplasmatic reticulum

The hypothesis that the final formation of 19 S thyroglobulin occurs only in the cisternae of the endoplasmatic reticulum is compatible with these findings Iodination of the protein is a separate process occurring only in the fully matured thyroid gland

1 Shepard T H J Clin Endocr 27 945 1967

R Riviere R Rappaport & D Comar (Hopital Frederic Joliot and Hopital des Enfants Malades Paris) *Thyroid function in hypopituitarism Reduction of the intrathyroidal organic iodine pool after TSH stimulation and suppressibility of residual thyroid function*

The possibility to correlate previous findings of a diminished response to prolonged TSH stimulation with a reduction of the intrathyroidal organic iodine pool was investigated in hypopituitary dwarfs During a seven days TSH stimulation test a $^{127}$I balance study was performed according to a previously published technique (1) We could calculate on the seventh day the extrathyroidal (E T P ) and intrathyroidal (I T P ) organic iodine pools Results were

| | Age (yrs) | Weight | Height | I T P | E T P |
|---|---|---|---|---|---|
| 1 Normal | 8 | 21 | 1 21 | 4060 | 1060 |
| 2 Normal | 14 | 31 | 1 44 | 3200 | 910 |
| 3 Normal | 16 | 35 | 1 46 | 040 | 1400 |
| 4 Idiopathic dwarfism | 4 | 11 | 0 87 | 13 0 | 460 |
| 5 Idiopathic dwarfism | 9 | 17 | 1 06 | 300 | 500 |
| 6 Idiopathic dwarfism | 10 | 22 | 1 13 | 240 | 960 |
| 7 Hypopituitarism | 14 | 21 | 1 12 | 840 | 360 |
| 8 Hypopituitarism | 12 | 19 | 1 11 | 495 | 375 |

Low I T P were found in two hypopituitary dwarfs and in one case of primordial growth retardation Diminished I T P corresponded in these cases with subnormal responses to TSH stimulation (hormonal PBI increment was below 4 $\mu g$/100 ml plasma) This was in agreement with the initial hypothesis

In five cases of pituitary insufficiency (including one with craniopharyngioma) the suppressibility of thyroid activity by 1 triiodothyronine (62 5 $\mu$g/m²/day for 10 days) was observed mean hormonal PBI decreased from 2 67 $\mu$g/100 ml (2 4 to 3 1 $\mu$g/100 ml) to 1 69 $\mu$g/100 ml (1 65 to 2 $\mu$g/100 ml) This was significant in all five cases compared to control values (before T3 5 67 ± 1 03 $\mu$g/100 ml after T3 2 62 ± 0 78 $\mu$g/100 ml $p <$ 0 001) T3 might act at different levels of the hypothalamic-thyroid axis Further investigation in cases due to a tumoral process are necessary to confirm the validity of this test in hypopituitary insufficiency

1 Rivière R *Bilans thyroïdiens de longue durée après administration d'iode radioactif* Rapport C E A R 2787 1965 Documentation Française—Paris

A Jost (Faculty of Sciences Paris intr by C G Bergstrand) *Hormonal factors in sex differentiation of the mammalian foetus*

The concept that the body is sexually bipotential during development has long been classical and has been substantiated by embryological and experimental studies In the so called hormonal theory of sex differentiation it was assumed that the genetic constitution (genetic sex) of the individual governs hormonal or humoral mechanisms which impose either maleness or femaleness on sexually neutral structure during successive steps (gonadal sex and later body sex)

As a matter of fact during the last two decades the experimental analysis of body sex differentiation showed that both sexes are not equal or equipotential as to their developmental trends and mechanisms In animal experiments it has been observed that in the absence of testes during several critical developmental stages many structures or systems develop along the feminine type, namely the genital tract the hypothalamic centers controlling the pituitary function the nervous structures mediating sex behaviour and the pattern of steroid metabolizing enzymes developing at puberty in the liver of the rat The ovary is unnecessary for the feminine differentiation of these structures in males femaleness has to be repressed and maleness imposed by the testes

The problem of *gonadal differentiation* and the mechanisms of action of the sex genes in bringing about ovarian or testicular organogenesis is still unsolved Free martins in cattle have long been considered to support the view that the initial sex differentiation of the gonad is hormonally controlled Actually free martins first develop ovaries which in a secondary developmental phase stop growing and become atrophic This atrophy cannot be duplicated by synthetic androgens acting on female fetuses it must result from some other agent

In Witschi s classical concept the undifferentiated gonadal anlage is made of two components (cortex and medulla) which produce antagonistic inductors Many observations could as well be understood by assuming that only one inductor or active mechanism is involved in initiating gonadal sex differentiation namely a mechanism imposing testicular organogenesis on a primordium which otherwise slowly becomes an ovary When gonadal sexual differentiation starts a marked histological change occurs in the testes whereas ovaries are first characterized mainly by the fact that they do not become testes

It is tempting to hypothesize that throughout sexual differentiation in mammals maleness has to be actively imposed on a system which becomes feminine when escaping this control (more details will be found in a paper in press in the Proc Roy Soc )

J J Van Wyk & F S French (Department of Pediatrics University of North Carolina School of Medicine Chapel Hill N C intr by C G Bergstrand) *Pathophysiology of the syndrome of testicular feminization*

Since the first description of testicular feminization in 1815 numerous theories have been

advanced to explain the various manifestations of this condition These have included chromosomal defects errors in testosterone biosynthesis defects in testosterone catabolism excessive peripheral conversion of testosterone to estrogen inhibition of testosterone action by high estrogen levels and unresponsiveness of the peripheral tissues to testosterone Since these hypotheses are not necessarily mutually exclusive and because evidence supporting each of them has been advanced each hypothesis was examined in critical detail

Hormonal levels were measured in two patients during a control period during adrenal suppression during stimulation with human pituitary FSH during stimulation with FSH plus HCG and following castration Plasma levels and urinary excretion of testosterone and estrogens were measured during each period as well as urinary 17 ketosteroids 17 ketosteroid fractions pregnantriol and 17 OH corticosteroids In addition the levels of testosterone and estrogens were measured in seminal venous plasma and the excised testes were incubated with isotopically labelled progesterone 17-OH progresterone and testosterone Testosterone 4 $^{14}$C was given i v and its disappearance rate determined and metabolic products in urine (including phenolic steroids) measured Lastly the effect of large doses of testosterone on nitrogen phosphorus and citric acid balances and gonadotrophin excretion was determined These studies led to the conclusions that all of the clinical and biochemical features of TFS which have been described can be satisfactorily explained as the consequence of a single gene defect leading to the failure of peripheral tissues to respond to testosterone Mauvais Jarvis Bercovici & Gauthier (J C E & M 29 p 420 1969) and Northcott, Island & Liddle (J C E & M 29 p 477 1969) simultaneously reported that target tissue from patients with TFS is unable to produce the active androgen dihydrotestosterone from labelled testosterone by reduction of ring A New evidence has been obtained from our laboratory however which suggests that this is not the primary defect in TFS but is more probably another result of the primary defect A patient with TFS failed to respond metabolically (nitrogen phosphorus and citric acid balances) to dihydrotestosterone when given in dosages which produced marked effects in a suitable control patient

D Aarskog (Department of Pediatrics University of Bergen) *Clinical and cytogenetic aspects of male pseudohermaphroditism*

Some clinical and cytogenetic aspects of male pseudohermaphroditism are discussed in view of the finding in 52 cases of hypospadias

All patients except for a girl with a congenital adrenal hyperplasia were reared as males

In 5 cases there was a history of maternal progestin treatment in early pregnancy One of these patients had a peno-scrotal hypospadias whereas the other four had hypospadias of the penile type There was a relationship between the position of the urethral meatus and the week of gestation at which progestin was used Thus the position of the hypospadias in the individual case reflected the phase of normal urethral groove fusion at the time of treatment It is probable that the progestin may affect human fetal genital development by inhibiting the activity of fetal 3 $\beta$ dehydrogenase enzyme and thereby mimicking the genital anomalies observed in both sexes in congenital adrenal hyperplasia associated with 3 $\beta$-dehydrogenase deficiency

There were 16 patients with peno-scrotal hypospadias and in this group there was 3 cases with XX XY mosaicism and one XO/XY mosaic Among 5 patients with the scrotal variety of hypospadias there was one case with XO/XY mosaicism XX/XY mosaicism has been found in different intersex conditions The 3 cases in this study fall into a clinico-cytogenetic group which might be designated XX/XY male pseudohermaphroditism

In five cases of pituitary insufficiency (including one with craniopharyngioma) the suppressibility of thyroid activity by 1 triiodothyronine (62.5 μg/m²/day for 10 days) was observed: mean hormonal PBI decreased from 2.67 μg/100 ml (2.4 to 3.1 μg/100 ml) to 1.69 μg/100 ml (1.65 to 2 μg/100 ml). This was significant in all five cases compared to control values (before T3 5.67±1.03 μg/100 ml, after T3 2.62±0.78 μg/100 ml, $p<0.001$). T3 might act at different levels of the hypothalamic-thyroid axis. Further investigation in cases due to a tumoral process are necessary to confirm the validity of this test in hypopituitary insufficiency.

1. Rivière, R. Bilans thyroïdiens de longue durée après administration d'iode radio-actif. Rapport C.E.A.R. 2757, 1965. Documentation Française—Paris.

A. Jost (Faculty of Sciences, Paris, intr. by C. G. Bergstrand): *Hormonal factors in sex differentiation of the mammalian foetus*

The concept that the body is sexually bipotential during development has long been classical and has been substantiated by embryological and experimental studies. In the so called "hormonal theory" of sex differentiation it was assumed that the genetic constitution (genetic sex) of the individual governs hormonal or humoral mechanisms which impose either maleness or femaleness on sexually neutral structures during successive steps (gonadal sex and later body sex).

As a matter of fact, during the last two decades the experimental analysis of *body sex* differentiation showed that both sexes are not equal or equipotential as to their developmental trends and mechanisms. In animal experiments it has been observed that in the absence of testes during several critical developmental stages, many structures or systems develop along the feminine type, namely the genital tract, the hypothalamic centers controlling the pituitary function, the nervous structures mediating sex behaviour, and the pattern of steroid metabolizing enzymes developing at puberty in the liver of the rat. The ovary is unnecessary for the feminine differentiation of these structures; in males, femaleness has to be repressed and maleness imposed by the testes.

The problem of *gonadal differentiation* and the mechanisms of action of the sex genes in bringing about ovarian or testicular organogenesis is still unsolved. Free martins in cattle have long been considered to support the view that the initial sex differentiation of the gonad is hormonally controlled. Actually, free martins first develop ovaries which in a secondary developmental phase stop growing and become atrophic. This atrophy cannot be duplicated by synthetic androgens acting on female fetuses; it must result from some other agent.

In Witschi's classical concept the undifferentiated gonadal anlage is made of two components (cortex and medulla) which produce antagonistic inductors. Many observations could as well be understood by assuming that only one inductor or active mechanism is involved in initiating gonadal sex differentiation, namely a mechanism imposing testicular organogenesis on a primordium which otherwise slowly becomes an ovary. When gonadal sexual differentiation starts, a marked histological change occurs in the testes, whereas ovaries are first characterized mainly by the fact that they do not become testes.

It is tempting to hypothesize that throughout sexual differentiation in mammals, maleness has to be actively imposed on a system which becomes feminine when escaping this control (more details will be found in a paper in press in the Proc. Roy. Soc.).

J. J. Van Wyk & F. S. French (Department of Pediatrics, University of North Carolina School of Medicine, Chapel Hill, N.C., intr. by C. G. Bergstrand): *Pathophysiology of the syndrome of testicular feminization*

Since the first description of testicular feminization in 1815, numerous theories have been

Nathalie Josso (Unite de Recherches de Genetique Medicale Hopital des Enfants Malades Paris intr by R Rappaport *Effect of a synthetic inhibitor of $\Delta_5$ 3β hydroxy steroid dehydrogenase on the in vitro development of the Wolffian ducts of the rat fetus*

$\Delta_5$ 3β hydroxy steroid dehydrogenase is a key enzyme in synthesis of testosterone and as such plays a leading role in sex differentiation Genetic or experimental inactivation of this enzyme in vivo produces a form of adrenal hyperplasia characterized by the incomplete masculinization of the genitalia of the affected males However *in vivo* overproduction of *adrenal androgens hampers the study of the* effect on the genital tract of the inhibition of testosterone synthesis Organ culture of the genital tract of male rat fetuses aged 16 ½ days was therefore carried out

30 tracts were cultured three day in the presence of cyanoketone 6 10⁻⁶M an inhibitor of $\Delta$ 3β hydroxy steroid dehydrogenase 30 tracts were cultured without the inhibitor Furthermore 12 castrated male tracts were cultured in anhormonal medium and 9 in the presence of dehydroepiandrosterone (DHA) 10⁻⁶M At the end of the culture period some explants were frozen for histoenzymatical study of testicular $\Delta$ 3β hydroxy steroid dehydrogenase NADH tetrazolium reductase and gluco-6-phosphate dehydrogenase Other explants were fixed and serially sectioned for study of the Wolffian ducts

Leydig cells of *explants cultured in control* medium exhibited activity *of all three enzymes* and Wolffian ducts were well developed Wolffian ducts were not maintained in castrated tracts cultured *in control medium*

Leydig cells of *explants cultured in the presence* of cyanoketone contained only trace amounts of $\Delta_5$ 3β hydroxy steroid dehydrogenase activity Other enzymes were normal and Wolffian ducts were well maintained Castrated tracts cultured with DHA also had normal Wolffian ducts

It is suggested that the maintenance of the Wolffian ducts despite the inhibition of testicular $\Delta$ 3β hydroxy steroid dehydrogenase could be due to the secretion of DHA by the rat fetal testis

Eveline de Peretti & Bernadette Loras (I N S E R M Unite de recherches Endocriniennes chez l enfant Hopital Debrousse Lyon) *In vitro studies with testicular tissue from two children with male pseudo hermaphrodism*

Homogenates of testicular tissue obtained from 2 children with male pseudohermaphrodism were incubated for one hour in the presence of $\Delta$5 pregnenolone 7 ³H and progesterone-4 ¹⁴C

The radioactive metabolites produced were purified and identified following column partition chromatography chromatography in various paper and thin layer systems formation of derivatives and their recrystallisation to constant specific activity

The younger child aged 10 was of Prader type II There was no evidence of pubertal development and the response of the plasma testosterone levels to stimulation with HCG was very limited in contrast plasma DHA sulphate levels rose significantly In his case utilization of radioactive precursors was poor However 17 hydroxylase activity was marked since 24 of the progesterone was converted to 17 OH progesterone and 27 of the pregnenolone was converted to 17 OH pregnenolone C 17 – C 20 desmolase activity was important in the $\Delta$5 pathway since DHA and $\Delta$5 androstenediol together accounted for 20 of the pregnenolone added at the onset In contrast there was little evidence of desmolase activity in the $\Delta$4 pathway since only 2 of the radioactivity originally present in progesterone was recovered in testosterone and $\Delta$4 androstenedione Conversion of $\Delta$5-compounds into $\Delta$4-compounds was very limited suggesting a $\Delta$5 3β ol dehydrogenase defect

The second child aged 12 had the type III malformation with clinical evidence of the onset of puberty Plasma testosterone showed a marked response to stimulation with HCG In

Gertrud Murset, M Zachmann & A Prader (Universitats Kinderklinik, Zurich) *Girl with male external genitalia caused by virilizing adrenal tumour of the mother*

A seven year old normal looking healthy boy with penile urethra was admitted because of bilateral cryptorchidism At the operation female internal genital organs were found (uterus two Fallopian tubes and two ovaries verified by histological examination) Chromosomal analysis revealed a female karyotype (46 XX) Congenital adrenal hyperplasia could be excluded (17 Ketosteroids 2 0 mg/24 h pregnantriol 0 09 mg/24 h and no pregnanetriolone) No androgens or gestagens were given during pregnancy The mother showed in spite of regular menstrual cycles some signs of virilization (somewhat deep voice slight hirsutism baldness and slightly enlarged clitoris) Her daily steroid excretion was 17 KS 71 mg DHA 61 mg testosterone 28 $\mu$g pregnantriol 0 3 mg no pregnanetriolone Further investigation in the department of Internal Medicine by Professor A Labhart and coworkers revealed an adrenal tumour that could be removed The histological examination showed an adenoma of the adrenal gland On the 7th postoperative day the daily steroid excretion was 17 KS 4 9 mg DHA 0 06 mg testosterone 7 3 $\mu$g

To our knowledge this is the first case of complete virilization of a girl caused by an adrenal tumour of the mother

H Berger J Glatzl & H Gleispach (Universitats Kinderklinik Innsbruck intr by C G Bergstrand) *Hormone excretion of boys with Klinefelter s syndrom*

Studying the urinary excretion of androgens and pregnanes of healthy boys and girls we are very interested in analyses of the steroid pattern of children with abnormalities of the sex chromosomes For the differentiation between steroids produced by the adrenals and those secreted by the gonads we made the test which was first proposed by Lloyd After the collection of a 24 hour urine, the adrenals were stimulated by ACTH—second 24 hour urine — Following the administration of dexamethasone—third 24 hour urine— 4 doses of human chorionic gonadotropines were given each second day—fourth 24 hour urine— In all urines we measured the 17 KS and pregnanes by gas liquid-chromatography

A 4 1/2 month old baby caryotype XXY with many degenerative signs showed a normal steroid pattern

A 7 year old boy caryotype XXY with adipositas showed a high excretion of all steroids testosterone was excreted in an amount much higher than normal but it was only produced by the adrenals no gonadal testosterone production could be stimulated

An 11 year old boy caryotype XXYY with adipositas, bone age advanced for two years showed also a high excretion of steroids produced by the adrenals The excretion of testosterone was low no gonadal testosterone production could be stimulated

A 10 year-old boy caryotype XXY lean eunuchoid stature, had a normal hormone excretion the production of *gonadal* testosterone *could* be stimulated

A 13 year old boy caryotype XXY/XXXY lean eunuchoid stature showed a normal steroid pattern also in this case the production of gonadal testosterone *could* be stimulated

A 29 year old young man caryotype XXY showed a normal steroid pattern except a low excretion of testosterone also in this case we could stimulate a little the production of gonadal testosterone When we summarize we must say that we found in all cases a normal function of the adrenals and in all cases we could stimulate the gonadal production of androsterone and etiocholanolone however a gonadal testosterone production could only be stimulated in the two boys with a lean eunuchoid stature and in the young man

given by Vihko and coworkers only using ... amount of Tetrahydro-corticosterone were found in the disulphate fraction

3 Allo-THF monosulphate could be demonstrated in all urines in a quantity which explains the difference between methods *b* and *c* Our first results suggest the tendency that the younger a child the higher the proportion of allo-THF excreted as monosulphate

William Hamilton (University Department of Child Health Royal Hospital for Sick Children Yorkhill Glasgow) *The treatment of congenital adrenal hyperplasia with α amino glutethimide*

The accepted principle for treating CAH is to replace the defective adrenocortical production cortisol and aldosterone with synthetic corticosteroid in dose which will suppress adrenal androgen secretion but which will not prevent normal linear growth This is frequently difficult to achieve especially in the salt losing form of the disease and in those cases coming late to treatment Aminoglutethimide (Elipten ...) a drug marketed for the control of epilepsy has been found to be an inhibitor of 20 desmolase This action suggested its use as blocker of all adrenocortical hormones in cases of CAH thus preventing androgen production ... and allowing the administration of small physiological amounts of glucocorticosteroids which would not prevent growth A therapeutic regime has been devised using the of the normal diurnal rhythm of plasma cortisol thus prednisolone 1 mg is given late in the evening and elipten 250 mg twice or thrice by day Five patients who were ... on average amounts of glucocorticoids which maintained urinary steroids at acceptable levels have been treated for up to ... year Elipten is excreted largely unchanged but small quantities of metabolites of oxidation and reduction appear in the urine Neither of these compounds nor elipten itself interferes with the Zimmermann Reaction

*Results* On this regime 4 patients have shown acceleration of linear growth the bone age either being held static or advancing more slowly than linear growth In the fifth case an example of 11β hydroxylase deficiency with hypertension bone maturation has been hindered but growth has been negligible In this child elipten potentiated the hypotensive action of guanethidine which alone did not control the blood pressure of 160/100 mm Hg During the period of treatment urinary 17 oxosteroids 17 oxogenic steroids and total 17 hydroxycorticosteroids have been maintained within acceptable limits

B T Rudd T Mo hang R L Rosenfield & W R Eberlein (Institute of Child Health Birmingham and Endocrine Dept Children's Hospital of Philadelphia) *Competitive protein binding assays for plasma and urinary testosterone*

Two new methods for the measurement of plasma testosterone and urinary testosterone (glucuronide) are presented Urinary testosterone is assayed from 5 ml aliquots of urine Recovery of 4 $^{14}$C testosterone added to urine ($N = 12$) after two stage TLC chromatography and derivative (acetate) formation was 40 ± 11 Coefficient of variation (11 pairs) was 2 Values for urinary testosterone (males and females $N = 10$) collated well with values from a double isotopic procedure ($r = 0\ 96$ $p <$ 001 ) Some preliminary values under basal conditions for children adult males and females are presented Infants and children (1 day–8 ½ yrs $N = 6$) gave values of < 0 1–2 9 μg/day Females (21–45 yrs $N = 4$) 1 7–6 1 μg/day Males (22–34 yrs $N = 4$) 11 8–42 μg/day

Plasma testosterone is assayed in 3–5 ml aliquots of plasma employing tritiated (1–2$^{3}$H) testosterone as internal marker for calculation of recovery Recovery from plasma was 83 ± 8% after extraction and two stage TLC Precision for replicate samples was males 238

*his case* the results following incubation were quite different and were what one might expect in a normal subject Utilization of precursor steroids was excellent 17α hydroxylase activity was very high but there was no accumulation of the 17α hydroxylated metabolites Side-chain splitting by C17–C20 desmolase seemed to be as important in the Δ4pathway as in the Δ5 pathway since the Δ4 products androstenedione and testosterone accounted for 32 8% of the progesterone added while the Δ5 products DHA and Δ5 androstenediol accounted for 35 3% of the pregnenolone The *presence of Δ5 3β ol dehydrogenase activity* was illustrated in this second case by a significant tritium count in Δ4 androstenedione and in testosterone

P Pujol Amat J Esteban Altirriba J Vanrell A Tejero A Oriol Bosch J Ribas Mundo & J Prats (Dept of Obstetrics & Gynecology Hospital de San Pablo Instituto de *Maternologia Instituto de Genetica* Humana Barcelona and Catedra de Fisiologica Facultad de Medicina de Madrid intr by J M Francés) *Testicular feminizing syndrome and pure gonadal dysgenesis Report of cases and in vitro studies with testes homogenate*

Four patients with testicular feminizing syndrome and one patient with pure gonadal dysgenesis constitute the basis of this report Urinary steroid analyses were performed in one of the patients prior and after gonadectomy The most striking feature was the high levels of urinary estrogens which decreased after gonadectomy pointing to a gonadal origin of the estrogens However this could not be confirmed by the *in vitro* studies with these gonads A homogenate of the testicular tissue was incubated with 4-[14]C Progesterone and 7 [3]H Pregnenolone Accumulation of 17 OH Progesterone and Dehydroepiandrosterone out of Progesterone and Pregnenolone respectively was obtained Both Progesterone and Pregnenolone served as substrates for Testosterone synthesis These findings are in agreement with those reported by other authors The importance of clinical features and findings at laparotomy is stressed as the basis for the differential *diagnosis between testicular feminizing syn*drome and pure gonadal dysgenesis

W Blunck (Universitats Kinderklinik Hamburg Eppendorf) *Conjugation of corticosteroids in the urine of children*

The recovery of α ketolic metabolites of cortisol and cortisone (THF allo-THF THE) is changed by different methods of hydrolysis Using glucuronidase hydrolysis in the urine (method *a*) the recovery of allo THF is relatively low in the urine of children After conjugate extraction (KORNEL) and application of the same glucuronidase preparation (method *b*) the recovery of THF + allo THF + THE increases 1 2 fold the total increase is mostly due to higher allo THF recovery (1 99 fold) Following conjugate extraction glucuronidase sulphatase hydrolysis extraction of the liberated steroids and consecutive solvolysis (method *c*) the total recovery of THF + allo-THF + THE increases 1 37 fold of allo THF 2 4 fold as compared with method *a*

Using method c no difference between the ratio allo THF THF in children as compared to adults could be found statistically An alteration of the 5α reduction pathway in the catabolism of cortisol during adolescence seems to be unlikely whereas an alteration in the mode of conjugation more probable

By thin layer chromatography on silica gel impregnated with barbital (pH 8 4) the corticosteroidglucuronides disulphates and monosulphates are separated After estimation of the single metabolites in these 3 fractions the following results could be obtained

1 Most of the cortisol and corticosterone metabolites are excreted as glucuronides

2 The excretion of cortisol and corticosterone metabolites as disulphates must be minimal they could not be detected with our method Even after working up a total 24 hour urine using the Sephadex LH 20 column

therapy resulted in impaired pituitary adrenal function in six out of seven patients the re duced responsiveness affected the anterior pituitary or the adrenal cortex as well However the clinical importance of this side effect is still another question

M I New & M P Seaman (Cornell University Medical College New York N Y) *Secretion rates of cortisol and aldosterone precursors in various forms of congenital adrenal hyperplasia*

To elucidate the site of the enzyme deficiency in various forms of congenital adrenal hyperplasia a method has been devised for the simultaneous determination of the secretion rates of cortisol and aldosterone precursors Secretion rates of cortisol (F) 11-desoxycortisol (S) Corticosterone (B) 11-desoxycorticosterone (DOC) and aldosterone (aldo) were determined in 10 normal subjects children with simple virilizing adrenal hyperplasia (21 hydroxylase defect) hypertensive virilizing adrenal hyperplasia (11 hydroxylase defect) and a family with dexamethasone suppressible hyperaldosteronism under the following conditions normal low and high sodium (Na) diets administration of metyrapone dexamethasone and intravenous ACTH The *mean daily normal secretion rates* were F 7 5 mg/m S 26 mg/m B 2 2 mg/m DOC 0 055 mg/m Aldo 0 13 mg/m Changes in dietary Na altered only aldo secretion ACTH administration raised B and F secretion significantly Metyrapone increased *S and DOC* secretion more than ACTH but decreased B F and aldo secretion In *21 hydroxylase defect* the secretion rates of B F DOC and S do not increase appropriately with ACTH and aldo secretion shows a blunted increase with low Na diet Secretion of B and F were below normal under all conditions In *the 11 hydroxylase defect* the secretion rates of B and F are very low and do not increase with ACTH while the secretion rates of DOC and S are 100 × normal and increase further with ACTH and metyrapone Aldo secretion is very low and does not increase with Na deprivation In the *syndrome of dexamethasone suppressible hyperaldosteronism* the secretion rates of F and aldo precursors are normal Results confirm a deficiency of 21 hydroxylase in the simple form and a deficiency of 11 hydroxylase in the hypertensive form of adrenal hyperplasia and do not suggest an enzyme defect in dexamethasone suppressible hyperaldosteronism

H Stolecke (Kinderklinik des Klinikum Essen der Ruhr Universitat Bochum intr by Ruth Illig) *The relation between urinary free cortisol and urinary 17 OHCS determined as PORTER SILBER chromogens under basal conditions and after stimulation with ACTH*

Our investigation was made trying to demonstrate a correlation of free urinary cortisol and the urinary 17 OHCS determined as PORTER SILBER-chromogens The way of experimental conditions includes a basal value a value after a load with metyrapone and two or three values after stimulation with depot ACTH i m ("Cortrosyn Depot ORGANON ) The ACTH load was made in two different ways First a 3-day load beginning 48 hours after metyrapone test and in another collective a 1 day load without a foregoing metyrapone test The analyses were performed in samples of 24 hours urine of healthy infants aged 4 to 8 months

The results show a statistically significant dependence of PORTER SILBER-chromogens and free urinary cortisol in all investigated conditions Moreover there is evidence that after ACTH the values of free urinary cortisol raise faster than those of PORTER SILBER-chromogens making a so-called pocket lens-effect This may depend on a raised renal clearance for free cortisol on an altered binding of cortisol with transcortin and on an altered degradation in the liver On the other hand the dependence between free urinary cortisol and PORTER SILBER-chromogens after ACTH shows that the discussed extraadrenal effects

mμg% (N = 9) CV ± 14% Females 40 2 mμg* (N = 5) CV 14 7*%

Data is also presented from pilot studies on the effect of ACTH HCG and clomiphene on the levels of plasma and urinary testosterone in patients with poor and excessive production of testosterone

Rolf P Zurbrugg (University Children's Hospital Berne) *Hypothalamo pituitary adrenal function following long term corticosteroid therapy*

Long term corticosteroid therapy may cause suppression of endogenous pituitary-adrenal function which involves the danger of inadequate response to stress Since there is no uniformity in therapy with steroids there is also much controversy in regard to frequency and extent of this unwanted effect

For this reason we took advantage of the international collaborative study on renal diseases in children in which all patients suffering from nephrotic syndrome are subjected to exactly the same corticosteroid therapy in so far as dosage and duration of treatment are concerned Our aims were threefold first to grade suppression individually in terms of severity secondly to investigate suppressiveness at various levels along the hypothalamo pituitary adrenal axis, and thirdly to follow recovery from prolonged steroid medication

All patients were treated with prednisone 60 mg/m body surface divided in three equal daily dosages for one month followed by an intermittent dosage regimen 40 mg of prednisone/m on three consecutive days per week only for another month

It is now recognized that no single test can adequately assess the functional integrity of the entire hypothalamo pituitary adrenal axis Therefore a particular combination of dynamic tests were applied synacthen test was performed in order to measure adrenal cortical response to exogenous synthetic corticotrophin vasopressin test was carried out in order to quantitate endogenous ACTH release to insulin induced hypoglycemia the hypothalamus should respond by secreting the corticotrophin releasing factor finally the diurnal plasma cortisol rhythm reflects variation in the activity of even higher centers in the central nervous system

The magnitude of the response to synacthen demonstrates that the extent of adrenal suppression varies a great deal from patient to patient After one month of daily prednisone medication one out of three children who have never been on steroids before showed a complete adrenal suppression contrasting to a still normal response in the remaining two The same one month course of prednisone was also given to patients who had already been on prolonged steroid therapy at earlier occasions because of repeated relapses An almost completely reduced adrenocortical capacity was again found in one case whereas a still distinct response was demonstrated in the two other ones

When investigating the responsiveness at various levels along the pituitary adrenal axis we were quite impressed to see that a still normal adrenal response to exogenous ACTH does not exclude a depressed function at the pituitary level

Follow up studies gave evidence that severity of suppression and therefore recovery seems to be related to the duration of medication rather than to steroid dosage When relatively low dosages of prednisone are given but constantly for years recovery may drag on for many months or even years

From these preliminary studies one may say that after long term corticosteroid therapy comparable in regard to duration and dosage first an individual variation from complete suppression to unaffected adrenal function is found secondly pituitary function however may already be distinctly depressed when adrenal response to exogenous ACTH stimulation is still inconspicuous and thirdly when recovery is concerned duration of therapy probably seems to be more important than the daily dosage The standardized prednisone

H Schedewie F Haour & J Bertrand (INSERM Unite de recherches endocrinologiennes et metaboliques chez l enfant Hopital Debrousse Lyon and Universitats Kinderklinik Heidelberg) *Influence of short term alterations of circulating energy substrate on HGH secretion*

The aim of the present investigations was to study the influence of acute changes of circulating energy supply upon the regulation of GH secretion. The experiments were conceived to give an answer to the following questions 1) Is GH secretion induced promptly in situations of acute energy deficiency which we define as when a low blood sugar and low serum NEFA? 2) Can GH be stimulated repeatedly within short time intervals? 3) Is GH release blocked when energy is readily supplied by glucose?

Blood glucose serum NEFA HGH and cortisol were measured in 7 healthy young men and one woman during the course of three periods of alternating hyper and hypoglycaemia. The data obtained were rather unexpected with regard to the literature. They may be summarized in the following statements 1) A rapid fall in blood sugar and a hypoglycaemic state for a period of 60 min did not suffice to stimulate GH release in about one half of the insulin induced hypoglycaemias 2) When GH secretion did occur however it did not seem to be blocked by a 30-minute glucose infusion 3) During three periods of recurrent hypoglycaemia (serum NEFA also being low) GH output occurred with a certain regularity only towards the end of the third period. By this time 3 1/2 to 4 hours had elapsed since the first glucose load 4) The degree of neurogenic stress (as judged by the clinical symptoms of hypoglycaemia as well as measurement of the serum levels of cortisol) sometimes paralleled the GH secretion. However no consistent accordance was observed

The following conclusions may be drawn from these results. Under the conditions of our experiments no clear cut correlation was apparent 1) between the absolute level of circulating energy substrate (i e blood glucose and serum NEFA) on the one hand and GH secretion on the other 2) between the extent of blood sugar decrease and GH response 3) between neurogenic stress and GH release

It is suggested that GH secretion in humans above all follows an autonomous and individual rhythm independent of short term alterations of energy supply

C G Bergstrand

are a relatively constant pattern which modifies the ACTH action in the way of the pocket-lens effect Regarding our experimental conditions we can say that this effect seems to be a very sensitive sign for the action of ACTH on the adrenals for we have demonstrated a true adrenal stimulation by the determination of PORTER SILBER chromogens

Furthermore our investigation demonstrates the possibility to classify free urinary cortisol into the characteristic metabolites degrated from compounds which represent just a synthetic step in corticosteroidogenesis for example THS Pregnantriol Pregnandiol and other Our findings are the first step to prove our hypothesis that it i possible to detect the capacity of the adrenal enzymes involved in corticosteroidogenesis in indirect manner by forming ratio of those characteristic compounds

D M Cathro J Bertrand & Mary G Coyle (I N S E R M Unite de Recherches Hopital Debrousse Lyon and The Department of Obstetrics and Gynaecology University of Dundee) *Antenatal diagnosis of adrenocortical hyperplasia*

The foetal adrenal cortex i essentially involved in the high production of oestrogens whi h is characteristic of pregnancy It therefore seems logical that if the foetus has congenital adrenocortical hyperplasia this will be reflected in a raised excretion of oestrogens by the mother

Three women who have previously given birth to children affected by adrenocortical hyperplasia due to 21-hydroxylase deficiency have been studied during pregnancy One gave birth to an affected child and in her case the excretion of oestriol oestrone and oestradiol during pregnancy was significantly above normal The details have been published The results so far available for the other women suggest that when the foetus is normal the oestrogen excretion is also normal and that is not affected by the carrier state in th mother

1 Cathro D M Bertrand J & Coyle M G *Lancet I* 732 1969

H Helge, B Weber, J Hammerstein & F Neumann (Kinderklinken der Freien Universit Berlin und der Universitat Heidelberg Frauenklinik der Freien Universitat Berlin und Endokrinologische Abteilung der Schering AG Berlin) *Idiopathic precocious puberty Indication for therapeutic use of cyproterone acetate an antigonadotropic and antiandrogen substance?*

*Cyproterone acetate (CY)* in divided dai doses has been orally administered to 4 children with idiopathic precocious puberty On boy 9 years old was treated with 100 mg of the drug per day three girls 4 5 and 6 yea old respectively when treatment was starte received doses of 20 to 30 mg per day Th substance was given for more than a year the boy and two of the girls In the latter wh previously had been treated with chlormadinone acetate the therapeutic effects of the tw drugs were compared CY induced an inhibtion of androgen activity (skeletal maturatio and growth velocity) in addition to the sup pression of gonadotropin and oestrogen secr tion (breast development vaginal smear men struation)

During the last 6 months the growth rate all the patients was decreased to values belo the average for age (less than 5 cm/year) Th boy formerly complaining about frequer erections and pollutions was promptly relieve of those symptoms No adver e effects of C have been reported by the children or the parents up to 2 years of therapy

Data on changes in urinary excretion of go nadotropins oestrogens and 17 ketosteroids o and off therapy during a 3 month interruptio of treatment were presented

E Schedewie F Haour & J Bertrand (INSERM Unite de recherches endocriniennes et metaboliques chez l enfant Hopital Debrousse Lyon and Universitats Kinderklinik Heidelberg) *Influence of short term alterations of circulating energy substrate on HGH secretion*

The aim of the present investigations was to study the influence of acute changes of circulating energy supply upon the regulation of GH secretion The experiments were conceived to give an answer to the following questions 1) Is GH secretion induced promptly in situations of acute energy deficiency which we define as "low blood sugar and low serum NEFA"? 2) Can GH be stimulated repeatedly within short time intervals? 3) Is GH release blocked when energy is readily supplied by glucose?

Blood glucose serum NEFA HGH and cortisol were measured in 7 healthy young men and one women during the course of three periods of alternating hyper and hypoglycaemia The data obtained were rather unexpected with regard to the literature They may be summarized in the following statements 1) A rapid fall in blood sugar and a hypoglycaemic state over a period of 60 min did not suffice to stimulate GH release in about one half of the insulin induced hypoglycaemias 2) When GH secretion did occur however it did not seem to be blocked by a 30 minute gluco e infusion 3) During three periods of recurrent hypoglycaemia (serum NEFA al o being low) GH output occurred with a certain regularity only towards the end of the third period By this time 3 ½ to 4 hours had elapsed since the first glucose load 4) The degree of neurogenic stress (as judged by the clinical symptoms of hypoglycaemia as well as measurement of the serum levels of cortisol) sometimes paralleled the GH secretion However no consistent accordance was observed

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It is suggested that GH secretion in humans above all follows an autonomous and individual rhythm independent of short term alterations of energy supply

*C G Bergstran*

*Acta Pædiat Scand 58 674–676 1969*

# BOOK REVIEWS

P R Rickham & J H Johnston (eds) *Neonatal surgery* Butterworth & Co London 1969 633 pp £8 10s

The authors serve as senior surgeon and urological surgeon respectively of Alder Hey Children's Hospital in Liverpool A neonatal surgical centre serving the area of the Liverpool Regional Hospital Board North Wales and adjoining regions was organized in 1953 This monograph is based on the experience gained from over 3000 newborn infants with surgical lesions treated since 1953

General aspects on neonatal surgery are dealt with in the first part of the book covering the incidence and causation of congenital defects organization lay-out and equipment of a regional neonatal surgical service neonatal physiology and its effects on anesthesia operative techniques and pre and postoperative management This section is concluded by a thourough and considerate discussion of the ethical aspects of neonatal surgery in particular with reference to indications for surgical treatment in the presence of combined serious malformations mongolism and other forms of severe mental retardation

A second section comprises congenital anomalies and other surgical disorders in newborn infants according to localization The text is throughout clear concise and easily accessible with special emphasis on matters of practical importance for diagnosis and management The book is richly illustrated by excellent photographs X ray pictures and schematic drawings The reproduction of X ray pictures is however not always satisfactory The contents of this book reflect the vast personal experience of the authors besides each section is concluded by thourough and up-to date references

This book is the first monograph dealing with neonatal surgery It serves a useful purpose in a useful way and should not be missing from the library shelves of any Department of Pediatrics or Pediatric Surgery

*Th Ehrenpreis*

János Horányi *Entwicklungsanomalien der Bronchialgewebe und ihre klinische Bedeutung* Akadémiai Kiadó Budapest 1969 194 pp US $8 40

According to the preface by Professor Mester this book is intended not only for the specialists (bronchologists lung specialists chest surgeons internists and pathologists) but primarily for practitioners particularly pediatricians Dr Horanyi seems to be a pathologist and he has dissected 3358 surgical lung specimens in an number of conditions but mainly from cases with evidence of bronchial disease such as anomalies of the bronchial cartilage valve mechanisms in emphysema tuberculosis and bronchial carcinoma He also includes a chapter on bronchial adenoma under the heading of malformations of the bronchi The method of examination includes washing out of the bronchial tree injection of fixative in the bronchi and arteries After fixation he divides the lugn in two parts the one including the bronchial stub is carefully sectioned longitudinally to the course of the bronchi All this is done with the clinical tomograms and bronchograms at hand Finally he makes histologic sections

The result is a meticulous description often from case to case of the light microscopic structure of the walls of the bronchi and their surrounding tissues The author describes bronchial stenoses in several instances he finds peribronchial changes labelled arteriomas Particular interest is focused on the mucous glands In a series of 2687 specimens he finds these glands to be decreased in number in TB increased in pneumonia mostly normal in carcinoma and bronchioma The chapter on vascular anomalies deals mostly with the arterioma and only a brief reference is made to the anastomoses between the bronchial and pulmonary artery systems Apparently no specimens are included from cases of cardiovascular anomalies so the monograph leaves the reader unaware of the common intrapulmonary vascular changes in congenital heart disease

The text is accompanied by 144 black and white photographs some radiograms some gross photographs but the majority photomicrographs Most of the reproductions are of dubious quality to say the least To a pathologist they very rarely enlighten the text

The book will possibly be a source of reference for researchers in the field of bronchial anomalies for example the upper lobe syndrome

*Bjørn I Ivemark*

C E Ford & Harry Harris (eds) *New aspects of human genetics* British Medical Bulletin 25 no 1 London 1969 118 pp illus £2

In this issue of the *British Medical Bulletin* many of the leading geneticists and cytogeneticists of the world have accounted for different areas of progress in human genetics and cytogenetics

It can already be said that this issue is an extremely valuable contribution to the human genetics and

a-operetics literature and should certainly be perused by all those interested in this field

There are sixteen articles by 22 authors besides the introduction written by Professor L. S. Penrose. Among those that I would like to mention is that of Harry Harris "Enzyme and Protein Polymorphism". The author points out that there are many different variant forms of glucose 6 phosphate dehydrogenase. D J Weatherall describes the genetics of thalassaemias pointing out that there is an underproduction of haemoglobin due to reduced rates of synthesis. Geoffrey Dean discusses different forms of porphyria and C O Carter gives new insight into polygenic inheritance in his paper "Genetics of Common Disorders". W M Court Brown who died in 1968 and P G Smith have written one of the most interesting papers "Human Population Cytogenetics". These two authors report that the frequency of translocation heterozygotes in a normal population is not less than 0.3 per cent. P E Polani describes different chromosomal aberrations in detail and even correlates phenotypes while Patricia Jacobs in writes about structural abnormalities of the sex chromosomes. Moreover facts about Xg blood groups, mosaics and reciprocal translocations are also reported

I would like to conclude this review with the closing remarks of L. S. Penrose. "The real value of this work can only be appreciated by careful study of the papers which will be found to provide highly concentrated information"

*Bertil Hall*

F Linneweh (ed) *Fortschritte der Pädologie* Springer Verlag Berlin Heidelberg and New York 1968 Band 246 pp DM 98

The second volume of Fortschritte der Pädologie covers, like the first one mainly different biophysiological aspects of the perinatal period. The first papers concern neonatal neurology (e.g. neonatal development of the brain myelination and neonatal reflexes). The rest of the book deals with varying aspects of the pediatric field e.g. neonatal circulation, respiratory physiology, function of the brown fat tissue, perinatal metabolism of steroids, hydrolysis of carbohydrates in infancy and renal clearance. Some papers deal with subjects of controversial nature and it is a difficult task to tackle them in relatively short surveys. Just a few remarks will be made. In the paper on respiratory physiology (control of neonatal breathing) due consideration has not been given to the role of positive pressure ventilation of infants with asphyxia. It is not enough to give them high oxygen content to breath. Analeptics are generally considered as useless and even dangerous in asphyxia but the author seems to believe in the benefit of these drugs

The quality of the different papers in this compilation of the book is uneven but all of them offer extensive lists of references. Some of the papers e.g. the review of hydrolysis of carbohydrates, new aspects on neonatal circulation and some of the neurological papers are however of very good quality. The book should be a valuable complement to the pediatric library

*Hans Ahlström*

S Z Levine (ed) *Advances in pediatrics vol XV* Year Book Medical Publishers Inc Chicago Illinois 1968 288 pp $12.50

Volume XV of Advances in pediatrics contains six chapters as usual excellent reviews of modern paediatric problems. Helfer and Pollock summarize "the battered child syndrome" and diagnosis and treatment of erythroblastosis in the fetus is reviewed by Liley. Tetanus of the newborn is to day of minor importance in the western countries but is still a major health problem in the underdeveloped areas. Florence N Marshall describes the pathogenesis, symptomatology and treatment of this infection with special reference to experiences in Haiti. Our present knowledge of aldosterone in childhood is reviewed by New and Peterson and prevention of prematurity is discussed by Rauh. The last chapter and by far the largest is devoted to pyelonephritis. Various aspects of this important problem are presented by Riley. This volume does not really need any recommendation it is enough to say that it holds the same high standard as its predecessors of this series

*C G Bergstrand*

Lytt I Gardner (ed) *Endocrine and genetic diseases of childhood* W B Saunders Company Philadelphia and London 1969 1072 pp £14 6s

In the preface of this textbook the editor remarks that in the last years the understanding of the endocrine and genetic diseases of childhood has evolved rapidly and that there is an accentuated need to integrate this knowledge. The book is an attempt to present to the medical student, to the specialist in paediatrics and to the practitioner a source of information on the pathophysiology, diagnosis and therapy of endocrine and genetic disorders in the paediatric age group. More than fifty well known American and European specialists have contributed and the result is certainly impressive. In 1072 pages great parts of the paediatric field are covered and information which may be difficult to collect from different sources has been presented in an easy and concentrated way. The first half of the book is devoted to the endocrine disorders of childhood and adolescence and the other half to genetic diseases. It is of course a matter of opinion what ought to be included in a textbook of this type but sometimes the reader gets the impression that the size of the chapters has been decided in a rather arbitrary way. Different types of periodic paralysis are described

in twenty pages the classic haemophilia A in one page The treatment of Cushing's syndrome in children occupies one page and a half but the prophylactic use of the antihaemophiliac factor and the orthopaedic measures in haemophilia is not discussed at all The guiding principle of this book may also be questioned and it is somewhat difficult to understand why such disparate subjects as thyroid disorders mongolism inherited haemoglobin abnormalities neonatal jaundice and congenital sugar malabsorbtion should be treated in one volume In spite of this criticism Endocrine and genetic diseases of childhood can be highly recommended to those who want to enlarge their knowledge of two rapidly developing fields of paediatrics

*C C Bergstrand*

V Dubowitz *Developing and Diseased Muscle* SIMP Research Monograph No 2 107 pp W Heinemann Medical Books London 1968 30s

Skeletal muscle fibres both in animal muscle and in human muscle are of two types One type is rich in mitochondria and oxidative enzymes and poor in enzymes like phosphorylase This is the red fibre or type I fibre The other type of muscle fibre contains less mitochondria and less oxidative enzymes and more phosphorylase This is the white fibre or type II fibre

During recent years much work has been done in different laboratories to clarify the biochemical and histochemical differences between the two fibre types Muscle pathologists have investigated how the different fibre types react in neuromuscular diseas[e] So far the results of the histochemical studies [of] diseased muscle have not given answers to q[uestions] about the aetiology of the main muscle diseases e[g] the muscular dystrophies Histochemical meth[ods] have however been of help in the diagnosis of so[me] rare muscle diseases and at many laboratories sta[in]ing for several enzymes belongs to the routine pro[ce]dures in the evaluation of muscles biopsies

Some animal muscles contain only type I fib[res] ("slow" muscles) some consist both of type I a[nd] type II fibres ("fast" muscles) A muscle consti[sting] of both the fibre types can be changed into a mus[cle] with only type I fibres if it is innervated by a "sl[ow] muscle" nerve

The pattern with two distinct fibre types is chara[c]teristic for the mature adult muscle In som[e] sp[ecies] like the guinea pig the muscles are mature at birt[h] In other species like the rat the muscles do not rea[ch] maturity until 10–14 days after birth Human mus[cle] shows an adult pattern at birth The pattern wi[th] two distinct types of muscle fibres can be disti[n]guished in human foetal muscles from the 28th w[eek] on

Dubowitz has contributed much to our knowled[ge] on the histochemistry of muscle Many of the f[acts] mentioned above come from his investigations T[he] book gives a detailed account of his own studies [A] short review of the literature on the histochemistr[y] of muscle is also given The book is of interest t[o s]cientists in all fields of muscle research To clin[i]cians the book is of limited interest

*K G Henriksson*

## ERRATUM

In Table 3 Tuckler G B Familial Hypophosphatemic Vitamin D Resistant Rickets The Neonatal Period and Infancy Acta Paediat Scand 58 21 1969 the stub listing "Phosphate clearance" should be followed by "ml/min/1 73 m²" not by "mg P 24 hr"